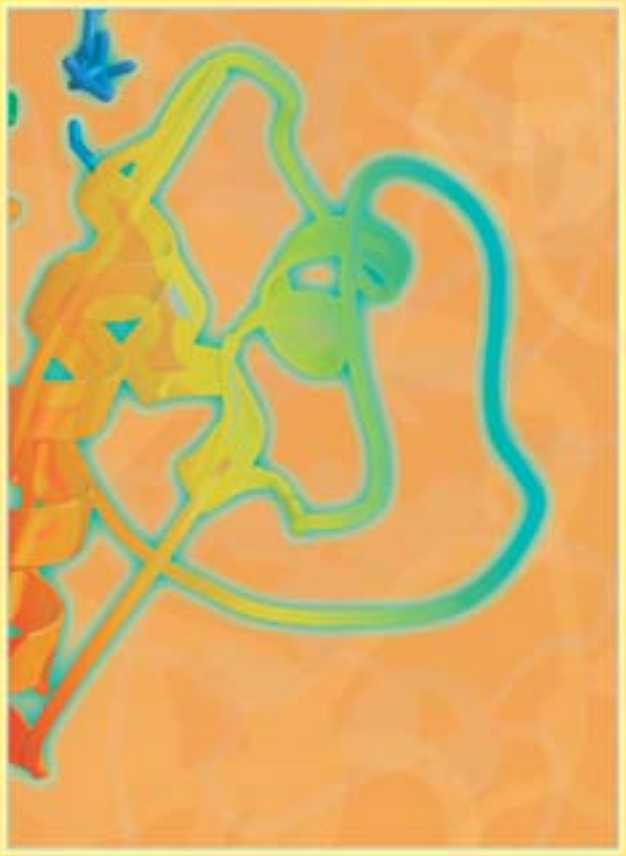
PIERCE

Protein Assay

Technical Handbook



PIERCE

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Quick Technical Summaries — Total Protein Assays

The BCA™ Protein Assay – Reducing Agent Compatible

Working Range	Characteristics/Advantages	Applications	Disadvantages	Interfering Substances[6]
Standard Protocol: *125-2,000 µg/ml*	Compatible with up to 5 mM DTT, 35 mM 2-mercaptoethanol or 10 mM TCEP No protein precipitation involved Sample volume only 25 µl Compatible with most detergents Significantly less (14–23%) protein:protein variation than Bradford-based methods Colorimetric method: measure	Allows the use of the superior BCA™ Assay in situations in which it is normally unable to be read No precipitation step means no worries about difficult-to-solubilize proteins	No microplate protocol is currently available Requires heating for color development	Compatible with all reducing agents and detergents found at concentrations routinely used in protein sample buffers

The BCA™ Protein Assay

Working Range	Characteristics/Advantages	Applications	Disadvantages	Interfering Substances[6]
Standard Protocol: *20-2,000 µg/ml* **Enhanced Standard Protocol:** *5-250 µg/ml* **Microplate Protocol:** *20-2,000 µg/ml*	Two stable reagents used to make one working reagent Working reagent stable for one week at room temperature Compatible with detergents Simple, easy to perform Less protein:protein variation than Coomassie dye methods Works with peptides (three amino acids or larger) Flexible incubation protocols allow customization of reagent sensitivity and working range	Adaptable for use with microplates[1] Determine the amount of IgG coated on plates[2] Measure the amount of protein covalently bound to affinity supports[3] Determine copper levels using a reagent formulated with BCA™ Reagent A[4] Use a microwave oven to develop the color in seconds[5]	Not compatible with thiols/reducing agents Requires heating for color development Not a true end-point assay	Reducing sugars and reducing agents Thiols Copper chelating agents Ascorbic acid and uric acid Tyrosine, cysteine and tryptophan 50 mM Imidazole, 0.1 M Tris, 1.0 M glycine

The Micro BCA™ Protein Assay

Working Range	Characteristics/Advantages	Applications	Disadvantages	Interfering Substances[6]
Standard Protocol: *60˚C for 60 minutes 0.5-20 µg/ml* **Microplate Protocol:** *37˚C for 120 minutes 1-20 µg/ml*	Three stable reagents used to make one working reagent Working reagent stable for 24 hours at room temperature Compatible with most detergents Simple, easy to perform Less protein:protein variation than BCA™, Coomassie dye or Lowry Methods Works with peptides (three amino acids or larger) Linear color response to increasing protein concentration	Suitable for determining protein concentration in very dilute aqueous solutions Adaptable for use with microplates[1]	More substances interfere at lower concentrations than with BCA™ Assay because the sample volume-to-reagent volume ratio is 1:1 60˚C water bath is needed	Reducing sugars and reducing agents Thiols Copper chelating agents Ascorbic acid and uric acid Tyrosine, cysteine and tryptophan 50 mM Imidazole, 0.1 M Tris, 1.0 M glycine

Quick Technical Summaries — Total Protein Assays

The Modified Lowry Protein Assay

Working Range	Characteristics/Advantages	Applications	Disadvantages	Interfering Substances[7]
Standard Protocol: *1-1,500 µg/ml*	Two-reagent system—shelf life of at least one year Two-step incubation requires precise sequential timing of samples Color response read at 750 nm Works with peptides (three amino acids or larger) Protein:protein variation similar to that seen with BCA™ Method Many authors have reported ways to deal with substances that interfere	Lowry method is the most cited protein assay in the literature Adaptable for use with microplates	Timed addition of Folin reagent adds complexity Longer total assay time Practical limit of about 20 samples per run	Detergents (cause precipitation) Thiols, disulfides Copper chelating reagents Carbohydrates including hexoseamines and their *N*-actyl derivatives Glycerol, Tris, Tricine, K^{+1} ions

Coomassie Plus – The Better Bradford™ Assay

Working Range	Characteristics/Advantages	Applications	Disadvantages	Interfering Substances[7]
Linear Range: IgG: *125-1,500 µg/ml* BSA: *125-1,000 µg/ml* **Standard Assay:** Sample-to-Reagent Ratio: 1:30 Typical Working Range: *100-1,500 µg/ml* **Micro Assay:** Sample-to-Reagent Ratio: 1:1 Typical Working Range: *1-25 µg/ml*	Simple/fast protocols Total preparation and assay time < 30 minutes One reagent system; stable for 12 months Ready-to-use formulation — no dilution or filtration needed Nearly immediate color development at room temperature Linear color response in standard assay (more accurate results) Color response sensitive to changes in pH Temperature dependence of color response Compatible with buffer salts, metal ions, reducing agents, chelating agents Low-odor formulation	Standard assay[8] Micro assay[9,10,11] Microplate format assay[12] Assay of protein solutions containing reducing agents[13] Quantitation of immobilized protein[14] Protein in permeabilized cells[15] $NaCNBH_3$ determination[16]	Less linear color response in the micro assay Effect of interfering substances more pronounced in the micro assay Protein dye complex has tendency to adhere to glass (easily removed with MeOH)[17] Protein must be >3,000 Da	Detergents[18]

Coomassie (Bradford) Protein Assay

Working Range	Characteristics/Advantages	Applications	Disadvantages	Interfering Substances[7]
Standard Assay: Sample-to-Reagent Ratio: 1:50 *100-1,500 µg/ml* **Micro Assay:** Sample-to-Reagent Ratio: 1:1 *1-25 µg/ml*	Simple-to-perform protocols One-reagent system, stable for 12 months Ready-to-use formulation No dilution or filtration needed Fast, nearly immediate color development at room temperature Total preparation and assay time < 30 minutes Typical protein:protein variation expected for a Coomassie dye-based reagent Color response sensitive to pH Temperature-dependent color response Compatible with buffer salts, metal ions, reducing agents, chelating agents	Standard assay[8] Micro assay[9,10,11] Microplate format assay[19] Assay of protein solutions containing reducing agents Cell line lysates[20] Protein recovery studies	Nonlinear color response More protein standard concentrations required to cover working range Micro assay has potential for interference Protein must be >3,000 Da	Detergents[18]

Pre-Diluted Protein Assay Standard Sets

Working Range	Characteristics/Advantages	Benefits
Working Range: *125-2,000 µg/ml*	Ready to use 3.5 ml each of seven standard curve data points within the working range Stable and sterile filtered 15-35 standard test tube assays or 175-350 microplate assays	No dilution series preparation Dramatically improved speed to result General utility standards for BCA™, Bradford and Lowry Assay methods More reliable quantitation Standard set is treated as you would treat the sample Unparalleled convenience Economical for microplate format assays

Quick Technical Summaries — References

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Total Protein Assays

Introduction

Protein quantitation is often necessary prior to handling protein samples for isolation and characterization. It is a required step before submitting protein samples for chromatographic, electrophoretic and immunochemical separation or analyses.

The most common methods for the colorimetric detection and quantitation of total protein can be divided into two groups based upon the chemistry involved. Protein assay reagents involve either protein-dye binding chemistry (coomassie/Bradford) or protein-copper chelation chemistry. Pierce offers seven colorimetric assays for detection and quantitation of total protein. They are all well-characterized, robust assays that provide consistent, reliable results. Collectively, they represent the state-of-the-art for colorimetric detection and quantitation of total protein.

Selection of the Protein Assay

When it is necessary to determine the total protein concentration in a sample, one of the first issues to consider is the selection of a protein assay method. The choice among available protein assays usually is based upon the compatibility of the method with the samples to be assayed. The objective is to select a method that requires the least manipulation or pre-treatment of the samples containing substances that may interfere with the assay.

Table 1. Pierce Protein Assay Reagents and their working ranges

Reagent	Protocol Used	Estimated Working Range
Coomassie (Bradford) Protein Assay	*Standard* tube or microplate *Micro* tube or microplate	100-1,500 µg/ml 1-25 µg/ml
Coomassie Plus – The Better Bradford™ Assay	*Standard* tube or microplate *Micro* tube or microplate	100-1,500 µg/ml 1-25 µg/ml
Coomassie Dry Protein Assay	*Standard* microplate	38-300 µg/ml
BCA™ Protein Assay – Reducing Agent Compatible	*Standard* tube	125-2,000 µg/ml
BCA™ Protein Assay	*Standard* tube or microplate *Enhanced* tube	20-2,000 µg/ml 5-250 µg/ml
Micro BCA™ Protein Assay	*Standard* tube *Standard* microplate	0.5-20 µg/ml 2-40 µg/ml
Modified Lowry Protein Assay	*Standard* protocol	1-1,500 µg/ml

Each method has its advantages and disadvantages (see previous pages). Because no one reagent can be considered to be the ideal or best protein assay method, most researchers have more than one type of protein assay reagent available in their labs.

If the samples contain reducing agents or copper chelating reagents, either of the ready-to-use liquid coomassie dye reagents (Coomassie [Bradford] Protein Assay or the Coomassie Plus – The Better Bradford™ Assay) would be excellent choices. If there is also a need to process many samples at one time, the Coomassie Dry Protein Assay Plates may be preferred.

The Modified Lowry Protein Assay offers all of the advantages of the original reagent introduced by Oliver Lowry in 1951 in a single, stable and ready-to-use reagent.

If the samples to be analyzed contain one or more detergents (at concentrations up to 5%), the BCA™ Protein Assay is the best choice. If the protein concentration in the detergent-containing samples is expected to be very low (< 20 µg/ml), the Micro BCA™ Protein Assay may be the best choice. If the total protein concentration in the samples is high (> 2,000 µg/ml), sample dilution can often be used to overcome any problems with known interfering substances.

Sometimes the sample contains substances that make it incompatible with any of the protein assay methods. The preferred method of dealing with interfering substances is to simply remove them. Pierce offers several methods for performing this function, including dialysis, desalting, chemical blocking and protein precipitation followed by resolubilization. This handbook focuses on the last two methods. Chemical blocking involves treating the sample with something that prevents the interfering substance from causing a problem. Protein precipitation causes the protein to fall out of solution, at which time the interfering buffer can be removed and the protein resolubilized. The chemical treatment method, like that used in the BCA™ Protein Assay – Reducing Agent Compatible, is generally preferred because, unlike protein precipitation, resolubilization of potentially hydrophobic proteins is not involved.

Selection of a Protein Standard

Selection of a protein standard is potentially the greatest source of error in any protein assay. Of course, the best choice for a standard is a highly purified version of the predominant protein found in the samples. This is not always possible or necessary. In some cases, all that is needed is a rough estimate of the total protein concentration in the sample. For example, in the early stages of purifying a protein, identifying which fractions contain the most protein may be all that is required. If a highly purified version of the protein of interest is not available or if it is too expensive to use as the standard, the alternative is to choose a protein that will produce a very similar color response curve with the selected protein assay method. For general protein assay work, bovine serum albumin (BSA) works well for a protein standard because it is widely available in high purity and is relatively inexpensive. Although it is a mixture containing several immunoglobulins, bovine gamma globulin (BGG) also is a good choice for a standard when determining the concentration of antibodies, because BGG produces a color response curve that is very similar to that of immunoglobulin G (IgG).

For greatest accuracy in estimating total protein concentration in unknown samples, it is essential to include a standard curve each time the assay is performed. This is particularly true for the protein assay methods that produce nonlinear standard curves. Deciding on the number of standards and replicates used to define the standard curve depends upon the degree of nonlinearity in the standard curve and the degree of accuracy required. In general, fewer points are needed to construct a standard curve if the color response is linear. Typically, standard curves are constructed using at least two replicates for each point on the curve.

Preparation of Standards

Use this information as a guide to prepare a set of protein standards. Dilute the contents of one Albumin Standard (BSA) ampule into several clean vials, preferably using the same diluent as the sample(s). Each 1 ml ampule of 2.0 mg/ml Albumin Standard is sufficient to prepare a set of diluted standards for either working range suggested. There will be sufficient volume for three replications of each diluted standard.

Preparation of Diluted Albumin (BSA) Standards for BCA™ Assay and BCA™ Reducing Agent-Compatible Assay

Dilution Scheme for Standard Test Tube Protocol and Microplate Procedure
(Working Range = 20-2,000 µg/ml)

Vial	Volume of Diluent	Volume and Source of BSA	Final BSA Concentration
A	0	300 µl of stock	2,000 µg/ml
B	125 µl	375 µl of stock	1,500 µg/ml
C	325 µl	325 µl of stock	1,000 µg/ml
D	175 µl	175 µl of vial B dilution	750 µg/ml
E	325 µl	325 µl of vial C dilution	500 µg/ml
F	325 µl	325 µl of vial E dilution	250 µg/ml
G	325 µl	325 µl of vial F dilution	125 µg/ml
H	400 µl	100 µl of vial G dilution	25 µg/ml
I	400 µl	0	0 µg/ml = Blank

Dilution Scheme for Enhanced Test Tube Protocol
(Working Range = 5-250 µg/ml)

Vial	Volume of Diluent	Volume and Source of BSA	Final BSA Concentration
A	700 µl	100 µl of stock	250 µg/ml
B	400 µl	400 µl of vial A dilution	125 µg/ml
C	450 µl	300 µl of vial B dilution	50 µg/ml
D	400 µl	400 µl of vial C dilution	25 µg/ml
E	400 µl	100 µl of vial D dilution	5 µg/ml
F	400 µl	0	0 µg/ml = Blank

Preparation of Diluted Albumin (BSA) Standards for Micro BCA™ Assay

Vial	Volume of Diluent	Volume and Source of BSA	Final BSA Concentration
A	4.5 ml	0.5 µl of stock	200 µg/ml
B	8.0 ml	2.0 ml of vial A dilution	40 µg/ml
C	4.0 ml	4.0 ml of vial B dilution	20 µg/ml
D	4.0 ml	4.0 ml of vial C dilution	10 µg/ml
E	4.0 ml	4.0 ml of vial D dilution	5 µg/ml
F	4.0 ml	4.0 ml of vial E dilution	2.5 µg/ml
G	4.8 ml	3.2 ml of vial F dilution	1 µg/ml
H	4.0 ml	4.0 ml of vial G dilution	0.5 µg/ml
I	8.0 ml	0	0 µg/ml = Blank

Preparation of Protein Standards for Coomassie Plus – The Better Bradford™ Assay and Coomassie (Bradford) Assay

Dilution Scheme for Standard Test Tube and Microplate Protocols
(Working Range = 100-1,500 µg/ml)

Vial	Volume of Diluent	Volume and Source of BSA	Final BSA Concentration
A	0	300 µl of stock	2,000 µg/ml
B	125 µl	375 µl of stock	1,500 µg/ml
C	325 µl	325 µl of stock	1,000 µg/ml
D	175 µl	175 µl of vial B dilution	750 µg/ml
E	325 µl	325 µl of vial C dilution	500 µg/ml
F	325 µl	325 µl of vial E dilution	250 µg/ml
G	325 µl	325 µl of vial F dilution	125 µg/ml
H	400 µl	100 µl of vial G dilution	25 µg/ml
I	400 µl	0	0 µg/ml = Blank

Dilution Scheme for Micro Test Tube or Microplate Protocols
(Working Range = 1-25 µg/ml)

Vial	Volume of Diluent	Volume and Source of BSA	Final BSA Concentration
A	2,370 µl	30 µl of stock	25 µg/ml
B	4,950 µl	50 µl of stock	20 µg/ml
C	3,970 µl	30 µl of stock	15 µg/ml
D	2,500 µl	2,500 µl of vial B dilution	10 µg/ml
E	2,000 µl	2,000 µl of vial D dilution	5 µg/ml
F	1,500 µl	1,500 µl of vial E dilution	2.5 µg/ml
G	5,000 µl	0	0 µg/ml = Blank

Preparation of Diluted Albumin (BSA) for Modified Lowry Assay

Dilution Scheme for Test Tube and Microplate Procedure
(Working Range = 1-1,500 µg/ml)

Vial	Volume of Diluent	Volume and Source of BSA	Final BSA Concentration
A	250 µl	750 µl of stock	1,500 µg/ml
B	625 µl	625 µl of stock	1,000 µg/ml
C	310 µl	310 µl of vial A dilution	750 µg/ml
D	625 µl	625 µl of vial B dilution	500 µg/ml
E	625 µl	625 µl of vial D dilution	250 µg/ml
F	625 µl	625 µl of vial E dilution	125 µg/ml
G	800 µl	200 µl of vial F dilution	25 µg/ml
H	800 µl	200 µl of vial G dilution	5 µg/ml
I	800 µl	200 µl of vial H dilution	1 µg/ml
J	1,000 µl	0	0 µg/ml = Blank

Total Protein Assays

Standards for Total Protein Assay

Bovine Serum Albumin Standard

The Pierce BSA Standard … the most relied-upon albumin standard for total protein determination measurements.

Ordering Information

Product #	Description	Pkg. Size
23209	**Albumin Standard Ampules, 2 mg/ml** Contains: Bovine Albumin in 0.9% NaCl solution containing sodium azide	10 x 1 ml
23210	**Albumin Standard, 2 mg/ml** Contains: Bovine Albumin in 0.9% NaCl solution containing sodium azide	50 ml

Bovine Gamma Globulin Standard

Easy-to-use, 2 mg/ml BGG solution. Ampuled to preserve product integrity. An excellent choice for IgG total protein determination. Recommended for Coomassie (Bradford) Assays.

Ordering Information

Product #	Description	Pkg. Size
23212	**Bovine Gamma Globulin Standard 2 mg/ml** Contains: Bovine Gamma Globulin Fraction II in 0.9% NaCl solution containing sodium azide	10 x 1 ml

Additional Mammalian Gamma Globulins for Standards:

Product #	Description	Pkg. Size
31878	**ImmunoPure® Mouse Gamma Globulin**	10 mg
31887	**ImmunoPure® Rabbit Gamma Globulin**	10 mg
31885	**ImmunoPure® Rat Gamma Globulin**	10 mg
31871	**ImmunoPure® Goat Gamma Globulin**	10 mg
31879	**ImmunoPure® Human Gamma Globulin**	10 mg

Pre-Diluted BSA and BGG Protein Assay Standard Sets

Construct a standard curve for most protein assay methods as fast as you can pipette.

Highlights:

- Stable and sterile filtered
- Ideal for BCA™ and Bradford-based protein assays
- Standard Curve Range: 125-2,000 µg/ml
- Seven data points within the range
- Sufficient materials to prepare 15-35 standard tube protocol curves or 175-350 standard microplate protocol curves running duplicate data points
- Convenient – no need to prepare a diluted standard series for each determination
- Consistent – no need to worry about variability in dilutions from day to day or person to person
- More reliable protein quantitation because of the assured accuracy of the concentrations of each standard
- Dramatically improved speed to result, especially with Bradford-based protein assays

Ordering Information

Product #	Description	Pkg. Size
23208	**Pre-Diluted Protein Assay Standards: Bovine Serum Albumin (BSA) Set** Diluted in 0.9% saline and preserved with 0.05% sodium azide Includes: 7 x 3.5 ml of standardized BSA solutions each at a specific concentration along a range from 125-2,000 µg/ml	Kit
23213	**Pre-Diluted Protein Assay Standards: Bovine Gamma Globulin Fraction II (BGG) Set** Diluted in 0.9% saline and preserved with 0.05% sodium azide Includes: 7 x 3.5 ml of standardized BGG solutions each at a specific concentration along a range from 125-2,000 µg/ml	Kit

Sample Preparation

Before a sample is analyzed for total protein content, it must be solubilized, usually in a buffered aqueous solution. The entire process is usually performed in the cold, with additional precautions taken to inhibit microbial growth or to avoid casual contamination of the sample by foreign debris such as hair, skin or body oils.

When working with tissues, cells or solids, the first step of the solubilization process is usually disruption of the sample's cellular structure by grinding and/or sonication or by the use of specially designed reagents (e.g., Poppers™ Cell Lysis Reagents) containing surfactants to lyse the cells. This is done in aqueous buffer containing one or more surfactants to aid the solubilization of the membrane-bound proteins, biocides (antimicrobial agents) and protease inhibitors. After filtration or centrifugation to remove the cellular debris, additional steps such as sterile filtration, removal of lipids or further purification of the protein of interest from the other sample components may be necessary.

Nonprotein substances in the sample that are expected to interfere in the chosen protein assay method may be removed by dialysis with Slide-A-Lyzer® Dialysis Cassettes or SnakeSkin® Dialysis Tubing, gel filtration with D-Salt™ Desalting Columns or Extracti-Gel™ D Detergent Removing Gel, or precipitation as in the Compat-Able™ Protein Assays or SDS-Out™ Reagent.

Protein:protein Variation

Each protein in a sample is unique and can demonstrate that individuality in protein assays as variation in the color response. Such protein:protein variation refers to differences in the amount of color (absorbance) obtained when the same mass of various proteins is assayed concurrently by the same method. These differences in color response relate to differences in amino acid sequence, isoelectric point (pI), secondary structure and the presence of certain side chains or prosthetic groups.

Table 2 (page 10) shows the relative degree of protein:protein variation that can be expected with the different Pierce protein assay reagents. This differential may be a consideration in selecting a protein assay method, especially if the relative color response ratio of the protein in the samples is unknown. As expected, protein assay methods that share the same basic chemistry show similar protein:protein variation. These data make it obvious why the largest source of error for protein assays is the choice of protein for the standard curve.

Total Protein Assays

For each of the six methods presented here, a group of 14 proteins was assayed using the standard protocol in a single run. The net (blank corrected) average absorbance for each protein was calculated. The net absorbance for each protein is expressed as a ratio to the net absorbance for BSA. If a protein has a ratio of 0.80, it means that the protein produces 80% of the color obtained for an equivalent mass of BSA. All of the proteins tested using the standard tube protocol with the BCA™ Protein Assay, the Modified Lowry Protein Assay, the Coomassie (Bradford) Assay and the Coomassie Plus – The Better Bradford™ Assay were at a concentration of 1,000 µg/ml.

Table 2. Protein:protein variation

	BCA™	Micro BCA™	Mod. Lowry	Coomassie (Bradford)	Coomassie Plus	Coomassie Dry	Bio-Rad (Bradford)
	Ratio	Ratio[1]	Ratio	Ratio	Ratio	Ratio[2]	Ratio
1. Albumin, bovine serum	1.00	1.00	1.00	1.00	1.00	1.00	1.00
2. Aldolase, rabbit muscle	0.85	0.80	0.94	0.76	0.74	0.44	0.97
3. α-Chymotrypsinogen	1.14	0.99	1.17	0.48	0.52	0.43	0.41
4. Cytochrome C, horse heart	0.83	1.11	0.94	1.07	1.03	0.57	0.48
5. Gamma Globulin, bovine	1.11	0.95	1.14	0.56	0.58	0.68	0.58
6. IgG, bovine	1.21	1.12	1.29	0.58	0.63	0.58	0.65
7. IgG, human	1.09	1.03	1.13	0.63	0.66	0.73	0.70
8. IgG, mouse	1.18	1.23	1.20	0.59	0.62	0.57	0.60
9. IgG, rabbit	1.12	1.12	1.19	0.37	0.43	0.45	0.53
10. IgG, sheep	1.17	1.14	1.28	0.53	0.57	0.71	0.53
11. Insulin, bov. pancreas	1.08	1.22	1.12	0.60	0.67	0.45	0.14
12. Myoglobin, horse heart	0.74	0.92	0.90	1.19	1.15	0.81	0.89
13. Ovalbumin	0.93	1.08	1.02	0.32	0.68	0.54	0.27
14. Transferrin, human	0.89	0.98	0.92	0.84	0.90	0.81	0.95
Avg. ratio	**1.02**	**1.05**	**1.09**	**0.68**	**0.73**	**0.63**	**0.60**
S.D.	**0.15**	**0.12**	**0.13**	**0.26**	**0.21**	**0.17**	**0.28**
CV	**14.7%**	**11.4%**	**11.9%**	**38.2%**	**28.8%**	**27%**	**46%**
Cost/Assay through Pierce	28¢	33¢	23¢	13¢	20¢	19¢	20¢
Cost/Assay through Competitor S	31¢	34¢	51¢	N/A	N/A	N/A	N/A

1. All of the proteins were tested using the standard tube protocol with the Micro BCA™ Protein Assay at a protein concentration of 10 µg/ml.
2. All of the proteins were tested using the standard protocol with the Coomassie Dry Protein Assay Plates at a protein concentration of 150 µg/ml.

This table is a useful guideline to estimate the protein:protein variation in color response that can be expected with each method. It does not tell the whole story. However, because the comparisons were made using a single protein concentration, it is not apparent that the color response ratio also varies with changes in protein concentration.

Compatible and Incompatible Substances

An extensive list of substances that have been tested for compatibility with each protein assay reagent can be found in the instruction booklet that accompanies each assay product. A copy can also be obtained from the Pierce web site.

In summary, the Coomassie (Bradford) and the Coomassie Plus – The Better Bradford™ Assays will tolerate the presence of most buffer salts, reducing substances and chelating agents, but they will not tolerate the presence of detergents (except in very low concentrations) in the sample. Strong acids or bases, and even some strong buffers, may interfere if they alter the pH of the reagent. The Coomassie Dry Protein Assay Plates are generally less tolerant of the presence of these substances because there is no dilution of the sample in the reagent.

The Modified Lowry Protein Assay is sensitive to the presence of reducing substances, chelating agents and strong acids or strong bases in the sample. In addition, the reagent will be precipitated by the presence of detergents and potassium ions in the sample.

The BCA™ Protein Assay is tolerant of most detergents but is sensitive to the presence of reducing substances, chelating agents and strong acids or strong bases in the sample. In general, the Micro BCA™ Protein Assay is more sensitive to the same substances that interfere with the BCA™ Protein Assay because less dilution of the sample is used.

Substances Compatible with Pierce Protein Assays

Concentrations listed refer to the actual concentration in the protein sample. A blank indicates that the material is incompatible with the assay; n/a indicates the substance has not been tested in that respective assay.

Substance	BCA™ – Reducing Agent Compatible Assay	BCA™ Assay	Micro BCA™ Assay	Modified Lowry Assay	Coomassie (Bradford) Assay	Coomassie Plus™ Assay
Detergents	(≤)	(≤)	(≤)	(≤)	(≤)	(≤)
Brij®-35	———	5.0%	5.0%	0.031%	0.125%	0.062%
Brij®-56	———	1.0%	1.0%	0.062%	0.031%	0.031%
Brij®-58	———	1.0%	1.0%	0.062%	0.031%	0.016%
CHAPS	10.0%	5.0%	1.0%	0.062%	5.0%	5.0%
CHAPSO	———	5.0%	5.0%	0.031%	5.0%	5.0%
Deoxycholic acid	———	5.0%	5.0%	n/a	0.05%	0.04%
Lubrol® PX	———	1.0%	1.0%	0.031%	0.125%	0.031%
Octyl glucoside	———	5.0%	1.0%	0.031%	0.5%	0.5%
Nonidet P-40	———	5.0%	5.0%	0.016%	0.5%	0.5%
Octyl β-thioglucopyranoside	10.0%	5.0%	5.0%	n/a	3.0%	3.0%
SDS (Lauryl)	10.0%	5.0%	5.0%	1.0%	0.125%	0.016%
Span® 20	———	1.0%	1.0%	0.25%	0.5%	0.5%
Triton® X-100	10.0%	5.0%	5.0%	0.031%	0.125%	0.062%
Triton® X-114	2.0%	1.0%	0.05%	0.031%	0.125%	0.062%
Triton® X-305	———	1.0%	1.0%	0.031%	0.5%	0.125%
Triton® X-405	———	1.0%	1.0%	0.031%	0.5%	0.25%
Tween®-20	10.0%	5.0%	5.0%	0.062%	0.062%	0.031%
Tween®-60	———	5.0%	0.5%	n/a	0.1%	0.025%
Tween®-80	———	5.0%	5.0%	0.031%	0.062%	0.016%
Zwittergent® 3-14	———	1.0%	———	n/a	0.025%	0.025%
Salts/Buffers	(≤)	(≤)	(≤)	(≤)	(≤)	(≤)
ACES, pH 7.8	———	25 mM	10 mM	n/a	100 mM	100 mM
Ammonium sulfate	———	1.5 M	———	———	1.0 M	1.0 M
Asparagine	———	1 mM	n/a	5 mM	10 mM	10 mM
Bicine, pH 8.4	———	20 mM	2 mM	n/a	100 mM	100 mM
Bis-Tris, pH 6.5	———	33 mM	0.2 mM	n/a	100 mM	100 mM
Borate (50 mM), pH 8.5	———	undiluted	1:4 dilution*	n/a	undiluted	undiluted
B-PER® Reagent	———	undiluted	1:10 dilution*	n/a	1:2 dilution*	1:2 dilution*
Calcium chloride in TBS, pH 7.2	———	10 mM	10 mM	n/a	10 mM	10 mM
Na-Carbonate/Na-Bicarbonate (0.2 M), pH 9.4	———	undiluted	undiluted	n/a	undiluted	undiluted
Cesium bicarbonate	———	0.1 M	0.1 M	50 mM	0.1 M	0.1 M
CHES, pH 9.0	———	100 mM	100 mM	n/a	100 mM	100 mM
Na-Citrate (0.6 M), Na-Carbonate (0.1 M), pH 9.0	———	1:8 dilution*	1:600 dilution*	n/a	undiluted	undiluted
Na-Citrate (0.6 M), MOPS (0.1 M) pH 7.5	———	1:8 dilution*	1:600 dilution*	n/a	undiluted	undiluted
Cobalt chloride in TBS, pH 7.2	———	0.8 mM	———	n/a	10 mM	10 mM
EPPS, pH 8.0	———	100 mM	100 mM	n/a	100 mM	100 mM
Ferric chloride in TBS, pH 7.2	———	10 mM	0.5 mM	n/a	10 mM	10 mM
Glycine	———	1 mM	n/a	100 mM	0.1 M	0.1 M
Guanidine•HCl	2 M	4 M	4.0 M	n/a	3.5 M	3.5 M
HEPES, pH 7.5	200 mM	100 mM	100 mM	1 mM	0.1 M	0.1 M
Imidazole, pH 7.0	50 mM	50 mM	12.5 mM	25 mM	200 mM	200 mM
MES, pH 6.1	100 mM	100 mM	100 mM	125 mM	100 mM	100 mM
MES (0.1 M), NaCl (0.9%), pH 4.7	———	undiluted	1:4 dilution*	n/a	undiluted	undiluted
MOPS, pH 7.2	———	100 mM	100 mM	n/a	100 mM	100 mM
Modified Dulbecco's PBS, pH 7.4	———	undiluted	undiluted	n/a	undiluted	undiluted
Nickel chloride in TBS, pH 7.2	———	10 mM	0.2 mM	n/a	10 mM	10 mM
PBS; Phosphate (0.1 M), NaCl (0.15 M), pH 7.2	———	undiluted	undiluted	n/a	undiluted	undiluted

n/a: not assayed
A blank indicates that the material is incompatible with the assay.
* Diluted with distilled/deionized water

Substances Compatible with Pierce Protein Assays (continued)

Substance	BCA™ – Reducing Agent Compatible	BCA™ Assay	Micro BCA™ Assay	Modified Lowry Assay	Coomassie (Bradford) Assay	Coomassie Plus™ Assay
Salts/Buffers (continued)	(≤)	(≤)	(≤)	(≤)	(≤)	(≤)
PIPES, pH 6.8	———	100 mM	100 mM	n/a	100 mM	100 mM
RIPA lysis buffer; 50 mM Tris, 150 mM NaCl, 0.5% DOC, 1% NP-40, 0.1% SDS, pH 8.0	———	undiluted	1:10 dilution*	n/a	1:10 dilution*	1:40 dilution*
Sodium acetate	———	0.2 M	0.2 M	0.2 M	180 mM	180 mM
Sodium azide	———	0.2%	0.2%	0.2%	0.5%	0.5%
Sodium bicarbonate	———	0.1 M	0.1 M	0.1 M	0.1 M	0.1 M
Sodium chloride	———	1.0 M	1.0 M	1.0 M	5.0 M	5.0 M
Sodium citrate, pH 4.8 (or pH 6.4)	———	200 mM	5 mM (16.7 mM)	n/a	200 mM	200 mM
Sodium phosphate	———	0.1 M	0.1 M	0.1 M	0.1 M	0.1 M
Tricine, pH 8.0	———	25 mM	2.5 mM	n/a	100 mM	100 mM
Triethanolamine, pH 7.8	———	25 mM	0.5 mM	n/a	100 mM	100 mM
Tris	50 mM	0.25 M	0.05 M	10 mM	2.0 M	2.0 M
TBS; Tris (25 mM), NaCl (0.15 M), pH 7.6	———	undiluted	1:10 dilution*	n/a	undiluted	undiluted
Tris (25 mM), Glycine (192 mM), pH 8.0	———	1:3 dilution*	1:10 dilution*	n/a	undiluted	undiluted
Tris (25 mM), Glycine (192 mM), SDS (0.1%), pH 8.3	———	undiluted	undiluted	n/a	1:2 dilution*	1:4 dilution*
Zinc chloride in TBS, pH 7.2	———	10 mM	0.5 mM	n/a	10 mM	10 mM
Reducing agents	(≤)	(≤)	(≤)	(≤)	(≤)	(≤)
N-acetylglucosamine in PBS, pH 7.2	———	10 mM	———	n/a	100 mM	100 mM
Ascorbic acid	———	———	———	1 mM	50 mM	50 mM
Catecholamines	———	———	———	n/a	n/a	n/a
Creatinine	———	———	———	n/a	n/a	n/a
Glucose	———	10 mM	1 mM	0.1 mM	1.0 M	1.0 M
Melibiose	———	———	n/a	25 mM	0.1 M	0.1 M
Potassium thiocyanate	———	3.0 M	n/a	0.1 M	3.0 M	3.0 M
Thiol-containing agents	(≤)	(≤)	(≤)	(≤)	(≤)	(≤)
Cysteine	———	———	———	1 mM	10 mM	10 mM
Dithioerythritol (DTE)	———	1 mM	———	———	1 mM	1 mM
Dithiothreitol (DTT)	5 mM	1 mM	———	———	5 mM	5 mM
2-Mercaptoethanol	35 mM	0.01%	1 mM	1 mM	1.0 M	1.0 M
TCEP	10 mM	———	———	———	———	———
Thimerosal	———	0.01%	———	0.01%	0.01%	0.01%
Chelating agents	(≤)	(≤)	(≤)	(≤)	(≤)	(≤)
EDTA	20 mM	10 mM	0.5 mM	1 mM	100 mM	100 mM
EGTA	———	———	———	1 mM	2 mM	2 mM
Sodium citrate, pH 4.8 (6.4)	100 mM	200 mM	5 mM (16.7 mM)	0.1 mM	200 mM	200 mM
Solvents/Misc.	(≤)	(≤)	(≤)	(≤)	(≤)	(≤)
Acetone	———	10%	1.0%	10%	10%	10%
Acetonitrile	———	10%	1.0%	10%	10%	10%
Aprotinin	———	10 mg/L	1 mg/L	10 mg/L	10 mg/L	10 mg/L
DMF	———	10%	1.0%	10%	10%	10%
DMSO	———	10%	1.0%	10%	10%	10%
Ethanol	———	10%	1.0%	10%	10%	10%
Glycerol (fresh)	———	10%	1.0%	10%	10%	10%
Guanidine•HCl	———	4.0 M	4.0 M	0.1 M	3.5 M	3.5 M
Hydrochloric acid	———	0.1 M	0.01 M	0.1 M	0.1 M	0.1 M
Leupeptin	———	10 mg/L	10 mg/L	10 mg/L	10 mg/L	10 mg/L
Methanol	———	10%	1.0%	10%	10%	10%
Phenol Red	———	———	n/a	0.01 mg/ml	0.5 mg/ml	0.5 mg/ml
PMSF	———	1 mM	1 mM	1 mM	1 mM	1 mM
Sodium hydroxide	———	0.1 M	0.05 M	0.1 M	0.1 M	0.1 M
Sucrose	40%	40%	4%	7.5%	10%	10%
TLCK	———	0.1 mg/L	0.1 mg/L	0.01 mg/L	0.1 mg/L	0.1 mg/L
TPCK	———	0.1 mg/L	0.1 mg/L	0.1 mg/L	0.1 mg/L	0.1 mg/L
Urea	4.0 M	3.0 M	3.0 M	3.0 M	3.0 M	3.0 M
o-Vanadate (sodium salt) in PBS pH 7.2	———	1 mM	1 mM	n/a	1 mM	1 mM

n/a: not assayed
A blank indicates that the material is incompatible with the assay.
* Diluted with distilled/deionized water

For more product information, or to download a product instruction booklet, visit *www.piercenet.com/path95n.*

Total Protein Assays

Time Considerations

The amount of time required to complete a total protein assay will vary for the seven colorimetric, total protein assay methods presented. To compare the amount of time required to perform each assay, all seven assays were performed using 20 samples and eight standards (including the blank). Each sample or standard was assayed in duplicate using the standard tube protocol (triplicate using the plate). The estimates include times for both incubation(s) and handling:

- Preparing (diluting) the standard protein in the diluent buffer (10 minutes)
- Organizing the run and labeling the tubes (5 minutes)
- Pipetting the samples and reagents (10 minutes for 56 tubes, 1 minute per plate)
- Mixing or incubating the tubes or plates (varies)
- Measuring the color produced (15 minutes for 56 tubes or 1 minute per plate)
- Graphing the standard curve, calculating, recording and reporting the results (30 minutes)

Table 3. Times required to assay 20 samples and 8 standards using the test tube procedure; handling times are considerably less using the microplate procedure

Method	Product #	Incubation Time	Total Assay Time
Coomassie Dry Plate Assay	23296	0 minutes	48 minutes
Coomassie Plus – The Better Bradford™ Assay	23236	10 minutes	80 minutes
Coomassie (Bradford) Assay	23200	10 minutes	80 minutes
BCA™ Assay	23225	30 minutes	100 minutes
Modified Lowry Assay	23240	10 minutes & 30 minutes	110 minutes
BCA™ Protein Assay – Reducing Agent Compatible	23250	45 minutes	115 minutes
Micro BCA™ Assay	23235	60 minutes	130 minutes

Calculation of Results

When calculating protein concentrations manually, it is best to use point-to-point interpolation. This is especially important if the standard curve is nonlinear. Point-to-point interpolation refers to a method of calculating the results for each sample using the equation for a linear regression line obtained from just two points on the standard curve. The first point is the standard that has an absorbance just below that of the sample and the second point is the standard that has an absorbance just above that of the sample. In this way, the concentration of each sample is calculated from the most appropriate section of the whole standard curve. Determine the average total protein concentration for each sample from the average of its replicates. If multiple dilutions of each sample have been assayed, average the results for the dilutions that fall within the most linear portion of the working range.

When analyzing results with a computer, use a quadratic curve fit for the nonlinear standard curve to calculate the protein concentration of the samples. If the standard curve is linear, or if the absorbance readings for your samples fall within the linear portion of the standard curve, the total protein concentrations of the samples can be estimated using the linear regression equation.

Most software programs allow one to construct and print a graph of the standard curve, calculate the protein concentration for each sample, and display statistics for the replicates. Typically, the statistics displayed will include the mean absorbance readings (or the average of the calculated protein concentrations), the standard deviation (SD) and the coefficient of variation (CV) for each standard or sample. If multiple dilutions of each sample have been assayed, average the results for the dilutions that fall in the most linear portion of the working range.

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Total Protein Assays

Bicinchoninic Acid (BCA™)-based Protein Assays

In 1985, Paul K. Smith, *et al.* introduced the BCA™ Protein Assay. Since then it has become the most popular method for colorimetric detection and quantitation of total protein. The BCA™ Protein Assay has a unique advantage over the Modified Lowry Protein Assay and any of the Coomassie dye-based assays — it is compatible with samples that contain up to 5% surfactants (detergents).

Briefly, the sample is added to the tube or plate containing the prepared BCA™ Working Reagent and after a 30-minute incubation at 37°C and cooling to room temperature, the resultant purple color is measured at 562 nm. The protocol is similar for the Micro BCA™ Protein Assay, except the ratio of sample volume to working reagent is different and the tubes are incubated for 60 minutes at 60°C.

Chemistry of BCA™-based Protein Assays

The BCA™ Protein Assay combines the well-known reduction of Cu^{2+} to Cu^{1+} by protein in an alkaline medium with the highly sensitive and selective colorimetric detection of the cuprous cation (Cu^{1+}) by bicinchoninic acid (Figure 1). The first step is the chelation of copper with protein in an alkaline environment to form a blue colored complex. In this reaction, known as the biuret reaction, peptides containing three or more amino acid residues form a colored chelate complex with cupric ions in an alkaline environment containing sodium potassium tartrate. This became known as the biuret reaction because a similar complex forms with the organic compound biuret (NH_2-CO-NH-CO-NH_2) and the cupric ion. Biuret, a product of excess urea and heat, reacts with copper to form a light blue tetradentate complex (Figure 2). Single amino acids and dipeptides do not give the biuret reaction, but tripeptides and larger polypeptides or proteins will react to produce the light blue to violet complex that absorbs light at 540 nm. One cupric ion forms a colored coordination complex with four to six nearby peptides bonds. The intensity of the color produced is proportional to the number of peptide bonds participating in the reaction. Thus, the biuret reaction is the basis for a simple and rapid colorimetric reagent of the same name for quantitatively determining total protein concentration. Since the working range for the biuret assay is from 5 to 160 mg/ml, the biuret assay is used in clinical laboratories for the quantitation of total protein in serum.

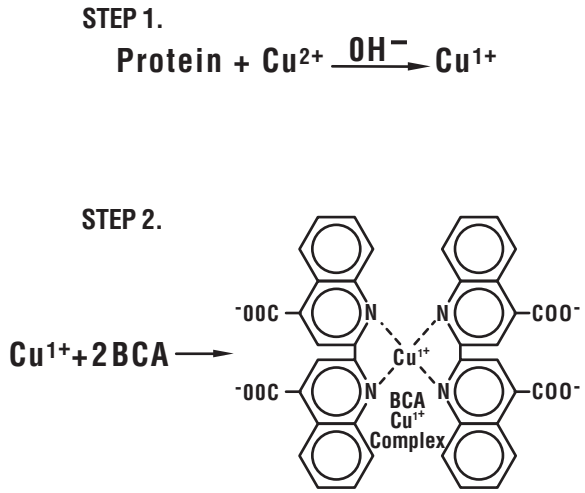


Figure 1. Reaction schematic for the bincinchoninic acid (BCA™)-containing protein assay.

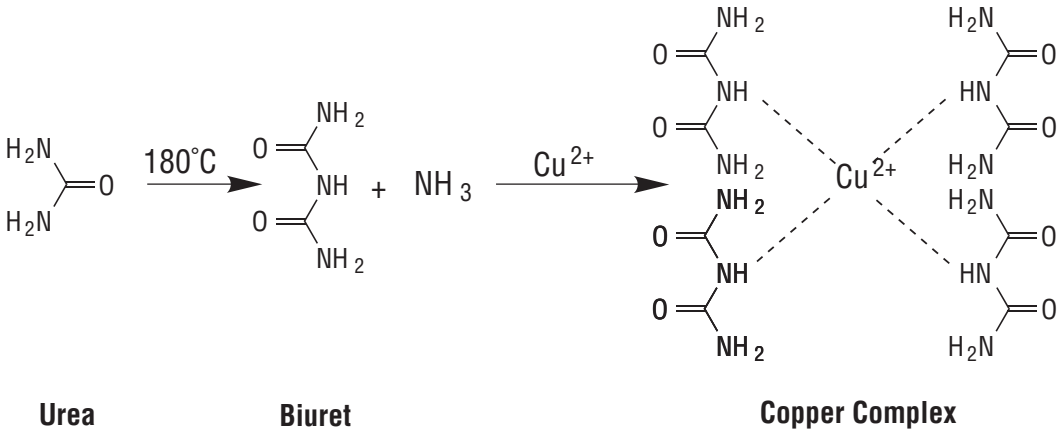


Figure 2. Biuret reaction schematic.

In the second step of the color development reaction, BCA™ Reagent, a highly sensitive and selective colorimetric detection reagent reacts with the cuprous cation (Cu^{1+}) that was formed in step 1. The purple colored reaction product is formed by the chelation of two molecules of BCA™ Reagent with one cuprous ion (Figure 1). The BCA™/Copper Complex is water-soluble and exhibits a strong linear absorbance at 562 nm with increasing protein concentrations. The purple color may be measured at any wavelength between 550-570 nm with minimal (less than 10%) loss of signal. The BCA™ Reagent is approximately 100 times more sensitive (lower limit of detection) than the biuret reagent. The reaction that leads to BCA™ Color Formation as a result of the reduction of Cu^{2+} is also strongly influenced by the presence of any of four amino acid residues (cysteine or cystine, tyrosine, and tryptophan) in the amino acid sequence of the protein. Unlike the Coomassie dye-binding methods that require a minimum mass of protein to be present for the dye to bind, the presence of only a single amino acid residue in the sample may result in the formation of a colored BCA™-Cu^{1+} Chelate. This is true for any of the four amino acids cited above. Studies performed with di and tripeptides indicate that the total amount of color produced is greater than can be accounted for by the color produced with each BCA™ Reagent-reactive amino acid. Therefore, the peptide backbone must contribute to the reduction of copper as well.

The rate of BCA™ Color Formation is dependent on the incubation temperature, the types of protein present in the sample and the relative amounts of reactive amino acids contained in the proteins. The recommended protocols do not result in end-point determinations, the incubation periods were chosen to yield maximal color response in a reasonable time frame.

Advantages of the BCA™ Protein Assay

The primary advantage of the BCA™ Protein Assay is that most surfactants (even if present in the sample at concentrations up to 5%) are compatible. The protein:protein variation in the amount of color produced with the BCA™ Protein Assay is relatively low, similar to that observed for the Modified Lowry Protein Assay (Table 2, page 10).

The BCA™ Protein Assay produces a linear response curve ($r^2 > 0.95$) and is available in two formulations based upon the dynamic range needed to detect the protein concentration of an unknown sample. The BCA™ Assay is less complicated to perform than the Lowry Protein Assay for both formulations. The standard BCA™ Protein Assay (Figure 3) detects protein concentrations from 20 to 2,000 µg/ml and is provided with Reagent A (carbonate buffer containing BCA™ Reagent) and Reagent B (cupric sulfate solution). A working solution (WS) is prepared by mixing 50 parts of BCA™ Reagent A with 1 part of BCA™ Reagent B (50:1, Reagent A:B). The working solution is an apple green color that turns purple after 30 minutes at 37°C in the presence of protein. The ratio of sample to WS used is 1:20. The Micro BCA™ Protein Assay (Figure 4) is more sensitive and has a narrower dynamic range of 0.1-25 µg/ml. To prepare the Micro BCA™ WS, three reagents (A, B and C) are mixed together at a ratio of 25 parts Micro Reagent A to 24 parts Micro Reagent B and 1 part Micro Reagent C. The Micro BCA™ WS is mixed with the sample or standard at a 1:1 volume ratio. The purple color response is read at 562 nm after 1 hour at 60°C.

Since the color reaction is not a true end-point reaction, considerable protocol flexibility is allowed with the BCA™ Protein Assay. By increasing the incubation temperature, the sensitivity of the assay can be increased. When using the enhanced tube protocol (incubating at 60°C for 30 minutes), the working range for the assay shifts to 5-250 µg/ml and the minimum detection level becomes 5 µg/ml.

Total Protein Assays

Both BCA™ Protein Assay formulations have less protein:protein variability than the Coomassie-based assays. The color response obtained for a seven point standard curve with the standard BCA™ Protein Assay using BSA or BGG standards shows less than a 20% variation between these two proteins (Figure 3). The Coomassie assay demonstrates >30% variation in the signal generated between BSA and BGG (Table 2, page 10). There is even less variation (<12%) when comparing these protein standards with the Micro BCA™ Protein Assay (Figure 4). In general, the BCA™ Protein Assay provides one of the most accurate measurements of protein concentration in biological samples, is detergent-compatible and simple to perform.

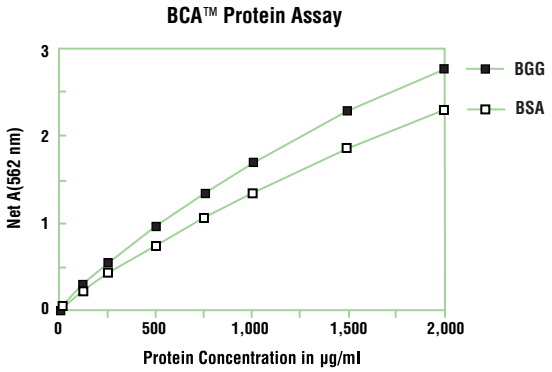


Figure 3. Color response curves obtained with the BCA™ Protein Assay using bovine serum albumin (BSA) and bovine gamma globulin (BGG). The standard tube protocol was performed and the color was measured at 562 nm.

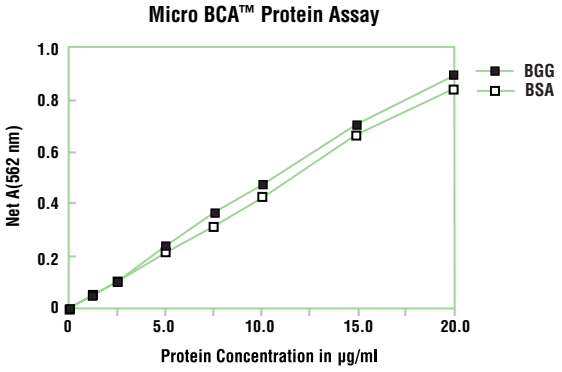


Figure 4. Color response curves obtained with the Micro BCA™ Protein Assay using bovine serum albumin (BSA) and bovine gamma globulin (BGG). The standard tube protocol was performed and the color was measured at 562 nm.

Disadvantages of the BCA™ Protein Assay

Substances that reduce copper will also produce color in the BCA™ Assay, thus interfering with the accuracy of the protein quantitation. Reagents that chelate the copper also interfere by reducing the amount of BCA™ Color produced with protein. Certain single amino acids (cysteine or cystine, tyrosine and tryptophan) will also produce color and interfere in BCA™ Assays.

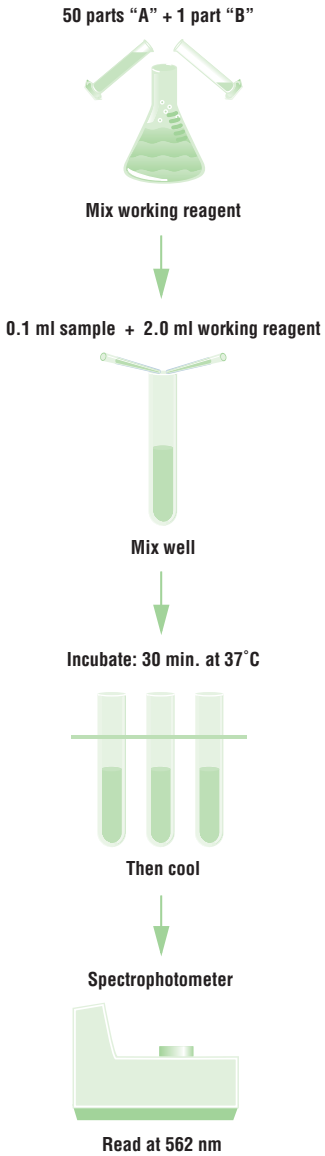
Total Protein Assays

The original BCA™ Protein Assay

Used in more labs than any other detergent-compatible formulation.

Highlights:

- Colorimetric method; read at 562 nm
- Compatible with most ionic and nonionic detergents
- Four times faster and easier than the classical Lowry method
- All reagents stable at room temperature for two years
- Working reagent stable for 24 hours
- Linear working range for BSA from 20 to 2,000 µg/ml
- Minimum detection level of 5 µg/ml with the enhanced protocol
- Convenient microplate or cuvette format
- Less protein:protein variation than dye-binding methods



BCA™ Protein Assay protocol.

Ordering Information

Product #	Description	Pkg. Size
23225	**BCA™ Protein Assay Kit** *Sufficient reagents to perform 500 standard tube assays or 5,000 microplate assays.* Includes: Reagent A Reagent B Albumin Standard (2 mg/ml)	Kit 2 x 500 ml 25 ml 10 x 1 ml ampules
23227	**BCA™ Protein Assay Kit** *Sufficient reagents to perform 250 standard tube assays or 2,500 microplate assays.* Includes: Reagent A Reagent B Albumin Standard (2 mg/ml)	Kit 1 x 500 ml 25 ml 10 x 1 ml ampules
23221	**BCA™ Protein Assay Reagent A** Contains: BCA™ and tartrate in an alkaline carbonate buffer	250 ml
23223	**BCA™ Protein Assay Reagent A** Contains: BCA™ and tartrate in an alkaline carbonate buffer	1,000 ml
23222	**BCA™ Protein Assay Reagent A** Contains: BCA™ and tartrate in an alkaline carbonate buffer	3.75 liter
23224	**BCA™ Protein Assay Reagent B** Contains: 4% $CuSO_4 \bullet 5H_2O$	25 ml
23230	**BCA™ Protein Assay Reagent A** Recrystallized purified powder	25 g
23228	**BCA™ Protein Assay Reagent A** Contains: BCA™ and tartrate in an alkaline carbonate buffer	500 ml

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Total Protein Assays

Micro BCA™ Protein Assay

Most sensitive BCA™ Formulation measuring dilute protein solutions from 0.5 to 20 µg/ml.

Highlights:

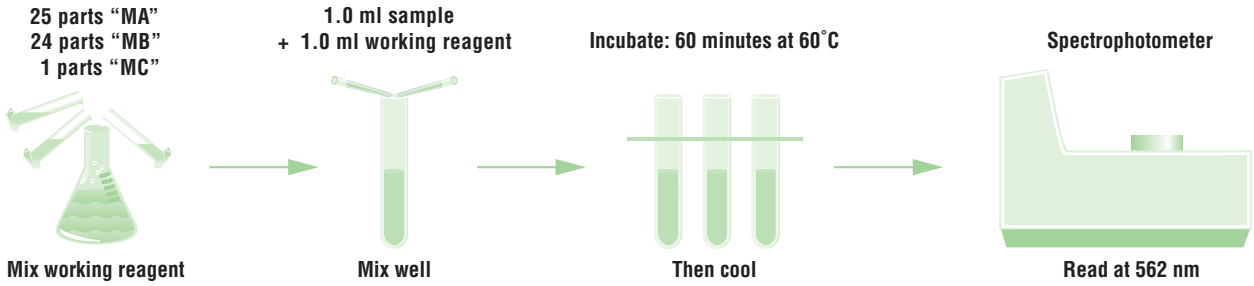
- Colorimetric method; read at 562 nm
- Compatible with most ionic and nonionic detergents
- A very sensitive reagent for dilute protein samples
- Linear working range for BSA: 0.5-20 µg/ml
- Less protein:protein variation than dye-binding methods
- All kit reagents stable at room temperature for two years
- Working reagent is stable for 24 hours
- Convenient microplate or cuvette format

References

Smith, P.K., *et al.* (1985). *Anal. Biochem.* **150 (1)**, 76-85.
Kang, D.E., *et al.* (2002). *Cell* **110**, 751-762.
Rawadi, G., *et al.* (1999). *J. Immunol.* **162**, 2193-2203.
Blum, D., *et al.* (2002). *J. Neurosci.* **22**, 9122-9133.
Paratcha, G., *et al.* (2003). *Cell* **113**, 867-879.

Ordering Information

Product #	Description	Pkg. Size
23235	**Micro BCA™ Protein Assay Kit** *Sufficient reagents to perform 480 standard tube assays or 3,200 microplate assays.* Includes: Micro Reagent A (MA) (Sodium carbonate, sodium bicarbonate, and sodium tartrate in 0.2 N NaOH) Micro Reagent B (MB) (4% BCA in water) Micro Reagent C (MC) (4% cupric sulfate pentahydrate in water Albumin Standard Ampules (2 mg/ml)	Kit 240 ml 240 ml 12 ml
23231	**Micro BCA™ Reagent A (MA)**	240 ml
23232	**Micro BCA™ Reagent B (MB)**	240 ml
23234	**Micro BCA™ Reagent C (MC)**	12 ml
23209	**Albumin Standard Ampules, 2 mg/ml** Contains: Bovine Albumin Fraction V in 0.9% NaCl solution containing sodium azide	10 x 1 ml



Micro BCA™ Protein Assay protocol.

Total Protein Assays

Coomassie Dye-based Protein Assays (Bradford Assays)

Use of Coomassie G-250 Dye in a colorimetric reagent for the detection and quantitation of total protein was first described by Dr. Marion Bradford in 1976. Both the Coomassie (Bradford) Protein Assay Kit (Product # 23200) and the Coomassie Plus – The Better Bradford™ Assay Kit (Product # 23236) are modifications of the reagent first reported by Dr. Bradford. Coomassie Dry Protein Assay Plates from Pierce contain Coomassie dye dried into each well.

Chemistry of Coomassie-based Protein Assays

In the acidic environment of the reagent, protein binds to the Coomassie dye. This results in a spectral shift from the reddish/brown form of the dye (absorbance maximum at 465 nm) to the blue form of the dye (absorbance maximum at 610 nm) (Figure 1). The difference between the two forms of the dye is greatest at 595 nm, so that is the optimal wavelength to measure the blue color from the Coomassie dye-protein complex. If desired, the blue color can be measured at any wavelength between 575 nm and 615 nm. At the two extremes (575 nm and 615 nm) there is a loss of about 10% in the measured amount of color (absorbance) compared to that obtained at 595 nm.

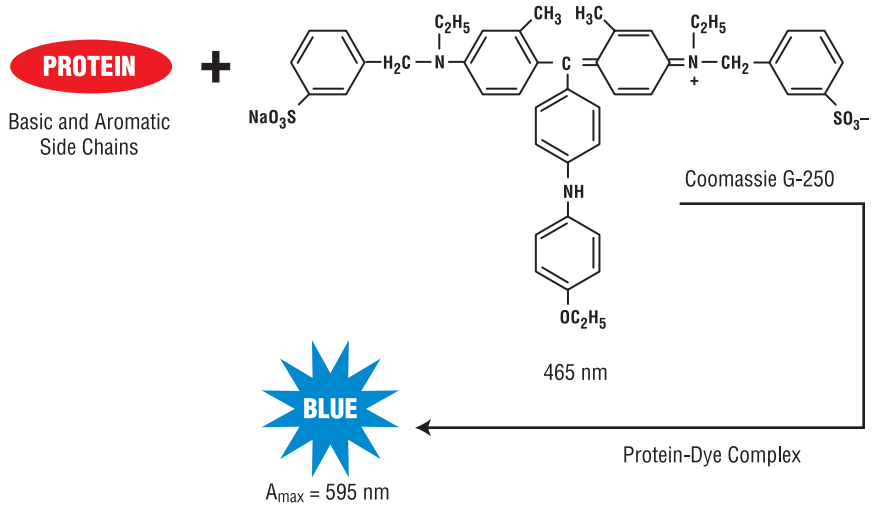


Figure 1. Reaction schematic for the Coomassie dye-based protein assays (the Coomassie [Bradford] Protein Assay, the Coomassie Plus – The Better Bradford™ Assay and the Coomassie Dry Protein Assay Plates).

Development of color in Coomassie dye-based protein assays has been associated with the presence of certain basic amino acids (primarily arginine, lysine and histidine) in the protein. Van der Waals forces and hydrophobic interactions also participate in the binding of the dye by protein. The number of Coomassie dye ligands bound to each protein molecule is approximately proportional to the number of positive charges found on the protein. Free amino acids, peptides and low molecular weight proteins do not produce color with Coomassie dye reagents. In general, the mass of a peptide or protein must be at least 3,000 daltons to be assayed with this reagent. In some applications this can be an advantage. The Coomassie (Bradford) Protein Assay has been used to measure "high molecular weight proteins" during fermentation in the beer brewing industry.

Advantages of Coomassie-based Protein Assays

Coomassie dye-binding assays are the fastest and easiest to perform of all protein assays. The assay is performed at room temperature and no special equipment is required. Briefly, for either the Coomassie (Bradford) Protein Assay or the Coomassie Plus – The Better Bradford™ Assay, the sample is added to the tube containing reagent and the resultant blue color is measured at 595 nm following a short room-temperature incubation. For the Coomassie Dry Protein Assay Plate, the sample is added directly to the well containing the dried reagent. After vigorous mixing, the plate is read immediately at 595 nm. The Coomassie dye-containing protein assays are compatible with most salts, solvents, buffers, thiols, reducing substances and metal chelating agents encountered in protein samples.

Disadvantages of Coomassie-based Protein Assays

The main disadvantage of Coomassie-based protein assays is their incompatibility with surfactants at concentrations routinely used to solubilize membrane proteins. In general, the presence of a surfactant in the sample, even at low concentrations, causes precipitation of the reagent. Since the Coomassie dye reagent is highly acidic, a small number of proteins cannot be assayed with this reagent due to their poor solubility in the acidic reagent. Also, Coomassie reagents result in about twice as much protein:protein variation as copper chelation based assay reagents (Table 2, page 10). In addition, Coomassie dye stains the glass or quartz cuvettes used to hold the solution in the spectrophotometer while the color intensity is being measured. (Cuvettes can be cleaned with strong detergent solutions and/or methanol washes, but use of disposable polystyrene cuvettes eliminates the need to clean cuvettes.)

General Characteristics of Coomassie-based Protein Assays (Bradford Assays)

Coomassie-based protein assays share a number of characteristics. The Coomassie (Bradford) Protein Assay produces a nonlinear standard curve. The Coomassie Dry Protein Assay Plates also produce a nonlinear standard curve, but over a smaller working range. The Coomassie Plus – The Better Bradford™ Assay has the unique advantage of producing a linear standard curve over part of its total working range. When using bovine serum albumin (BSA) as the standard, the Coomassie Plus – The Better Bradford™ Assay is linear from 125 to 1,000 µg/ml. When using bovine gamma globulin (BGG) as the standard, the Coomassie Plus – The Better Bradford™ Assay is linear from 125 to 1,500 µg/ml. The complete working range of the Coomassie Plus – The Better Bradford™ Assay covers the concentration range from 125 to 1,000 µg/ml for the tube protocol and from 1 to 25 µg/ml for the micro protocol (Figures 2-4).

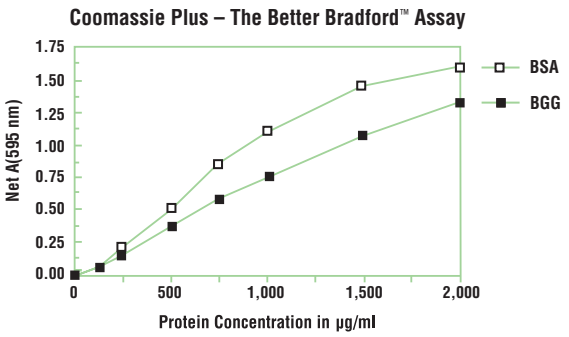


Figure 2. Color response curves obtained with Coomassie Plus – The Better Bradford™ Assay using bovine serum albumin (BSA) and bovine gamma globulin (BGG). The standard tube protocol was performed and the color was measured at 595 nm.

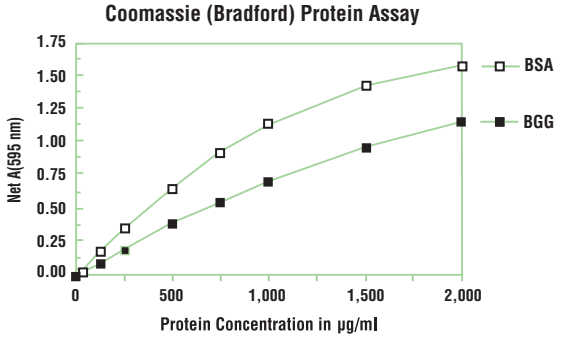


Figure 3. Color response curves obtained with Coomassie (Bradford) Protein Assay using bovine serum albumin (BSA) and bovine gamma globulin (BGG). The standard tube protocol was performed and the color was measured at 595 nm.

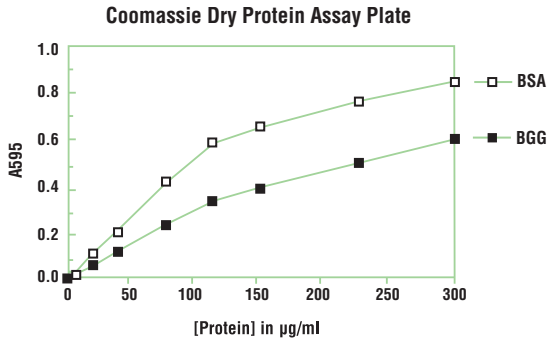


Figure 4. Color response curves obtained with Coomassie Dry Protein Assay Plates using bovine serum albumin (BSA) and bovine gamma globulin (BGG). The standard 96-well microplate protocol was performed and the color was measured at 595 nm.

Coomassie dye-based protein assays must be refrigerated for long-term storage. If Coomassie Dry Protein Assay Plates or ready-to-use liquid Coomassie dye reagents will be used within one month, either may be stored at ambient temperature (18-26°C). Coomassie protein assay reagent that has been left at room temperature for several months will have a lower color response, especially at the high end of the working range. Coomassie protein assay reagents that have been stored refrigerated must be warmed to room temperature before use. Using either cold plates or cold liquid Coomassie dye reagent will result in low absorbance values.

The ready-to-use liquid Coomassie dye reagents must be mixed gently by inversion just before use. The dye in these liquid reagents spontaneously forms loose aggregates upon standing. These aggregates may become visible after the reagent has been standing for as little as 60 minutes. Gentle mixing of the reagent by inversion of the bottle will uniformly disperse the dye. After binding to protein, the dye also forms protein-dye aggregates. Fortunately, these protein-dye aggregates can be dispersed easily by mixing the reaction tube. This is common to all liquid Coomassie dye reagents. Since these aggregates form relatively quickly, it is also best to routinely mix (vortex for 2-3 seconds) each tube or plate just before measuring the color.

Total Protein Assays

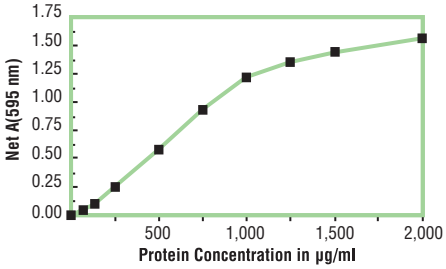
Coomassie Plus – The Better Bradford™ Protein Assay

As fast as the original Coomassie Assay, with increased accuracy … the high-performance Bradford reagent.

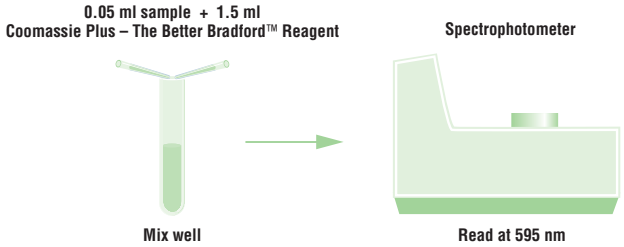
- **Easier, quicker preparation**
  Working reagent is ready to use. No tedious dilution, no filtration of a dye concentrate and no mess to clean up.
- **Lower cost per assay**
  Just 20¢ per sample with the standard protocol, 12¢ per sample with the micro protocol and 4¢ per sample with the microplate protocol.
- **Faster assay**
  Total assay time is less than 10 minutes!
- **More accurate results**
  Substantially increased linearity of response, and only half the expected protein:protein variation of other commercial formulations.

Highlights:

- Detects protein concentrations from 1 to 1,500 µg/ml
- Ready-to-use dye-binding reagent formulation
- Fast (almost immediate) color development read at 595 nm
- Compatible with reducing sugars, reducing substances and thiols
- Refrigerated reagent is stable for up to two years
- Superior linear response over the range of 125-1,500 µg/ml
- Convenient microplate or cuvette format
- Micro protocol useful for protein concentrations from 1 to 25 µg/ml



Typical color response curve for BSA using the Coomassie Plus – The Better Bradford™ Protein Assay Reagent.



Coomassie Plus – The Better Bradford™ Assay protocol. The protocol is simple, fast and very easy to perform.

Ordering Information

Product #	Description	Pkg. Size
23236	**Coomassie Plus – The Better Bradford™ Assay Kit** *Sufficient reagents to perform 630 standard assays or 3,160 microplate assays.* Includes: Coomassie Plus Protein Assay Reagent Albumin Standard (2 mg/ml)	Kit 950 ml 10 x 1 ml ampules
23238	**Coomassie Plus – The Better Bradford™ Reagent** *Sufficient reagents to perform 200 standard assays or 1,000 microplate assays.* Albumin Standard not included.	300 ml

Related Pierce Products:

Product #	Description	Pkg. Size
23239	**Coomassie Plus Compat-Able™ Protein Assay Kit**	Kit

Compatible Substances

Reagents compatible with Coomassie Plus – The Better Bradford™ Assay using the standard protocol. Interferences may be observed at the stated concentration when using the Micro Assay Procedure.

Ammonium Sulfate	1.0 M	2-Mercaptoethanol	1.0 M
Azide	0.5%	MES	100 mM
Brij®-56	0.03%	NaCl	5.0 M
Brij®-35	0.06%	NaOH	0.1 M
Brij®-58	0.016%	NP-40	0.5%
CHAPS	5.0%	SDS	0.016%
CHAPSO	5.0%	Sucrose	10.0%
Citrate	200 mM	Tris	2.0 M
EDTA	100 mM	Triton® X-100	0.06%
Glucose	1.0 M	Triton® X-114	0.06%
Glycine	0.1 M	Triton® X-405	0.25%
Guanidine•HCl	3.5 M	Tween®-20	0.03%
HCl	0.1 M	Tween®-80	0.016%
KSCN	3.0 M	Urea	3.0 M

References
Bradford, M. (1976). *Anal. Biochem.* **72**, 248-254.
Glover, B.P. and McHenry, C.S. (2001). *Cell* **105**, 925-934.
Kagan, A., *et al.* (2000). *J. Biol. Chem.* **275**, 11241-11248.
Goel, R., *et al.* (2002). *J. Biol. Chem.* **277**, 18640-18648.

Total Protein Assays

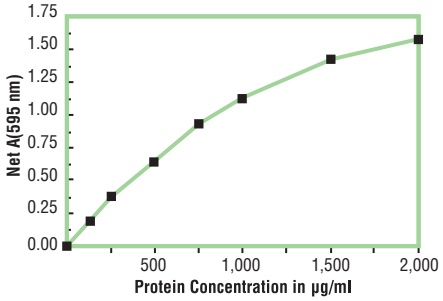
Coomassie (Bradford) Protein Assay

The Bradford method workhorse … ready-to-use, allowing total protein determination in seconds!

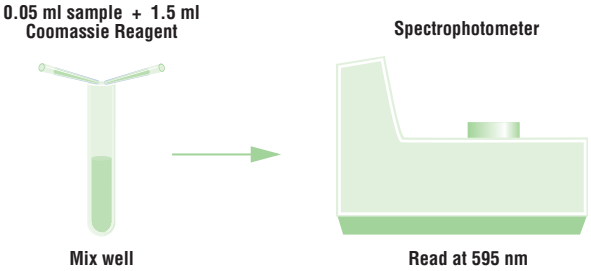
This ready-to-use formulation more closely resembles in performance, the reagent published by Bradford.[1] It demonstrates the typical assay characteristics known for Coomassie dye-based formulations.[2]

Highlights:

- Ready-to-use dye-binding reagent formulation
- Fast (almost immediate) color development; read at 595 nm
- Compatible with reducing substances and chelating agents
- Refrigerated reagent is stable for 12 months
- Determine protein concentration from 100 to 1,500 µg/ml
- Micro method for the range of 1 to 25 µg/ml
- Convenient microplate or cuvette format



Coomassie (Bradford) Protein Assay Reagent: typical color response curve for BSA.



Coomassie (Bradford) Protein Assay protocol.

Ordering Information

Product #	Description	Pkg. Size
23200	**Coomassie (Bradford) Protein Assay Kit** (Ready-to-use Coomassie Blue G-250 based reagent) *Sufficient reagents to perform 630 standard tube assays or 3,800 microplate assays.* Includes: Coomassie Protein Assay Reagent Albumin Standard Ampules (2 mg/ml)	Kit 950 ml 10 x 1 ml

References

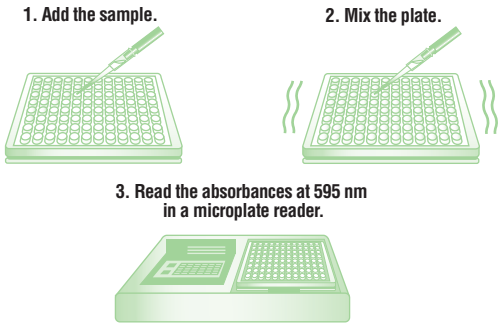
1. Bradford, M. (1976). *Anal. Biochem.* **72**, 248-254.
2. VanKley, H. and Hale, S.M. (1977). *Anal. Biochem.* **81**, 485-487.
   Messenger, M.M., *et al.* (2002). *J. Biol Chem.* **277**, 23054-23064.

Coomassie Dry Protein Assay Plates

Simplifying total protein analysis for the high-volume analyst.

Highlights:

- Working Range – 38 mg/ml-300 mg/ml (38 mg/well-30.0 mg/well)
- Coefficient of variation – 0.27
- Lowest demonstrated protein:protein variation of any homemade or commercial Coomassie dye-based formulation tested
- Sealed strip well plates – allows selection of as many or as few wells as needed for a specific total protein sample run
- Packaged to ensure plate integrity between uses



Coomassie Dry Protein Assay Plates protocol.

Ordering Information

Product #	Description	Pkg. Size
23296	**Coomassie Dry Protein Assay Plates** *Trial size contains sufficient materials to perform 192 individual assays.*	2 x 96 well plate pack
23596	**Coomassie Dry Protein Assay Plates** *Sufficient material to perform 480 individual assays.*	5 x 96 well

References

1. Yamada, N.A., *et al.* (2003). *Mutagenesis* **18**, 277-282.
2. Yamada, N.A. and Farber, R.A. (2002). *Cancer Res.* **62**, 6061-6064.

For more product information, or to download a product instruction booklet, visit *www.piercenet.com/path95n.*

Overcoming Interfering Substances

Virtually every protein detection method known exhibits sensitivity to the presence of particular reagents in the protein sample. Proteins are typically found in solutions that contain detergents, buffer salts, denaturants, reducing agents, chaotropic agents and/or anti-microbial preservatives. These additives may affect the results of an assay. When a component of a protein solution artificially increases or decreases the signal of any assay, the component is considered to be an interfering substance.

Interfering substances can affect the protein assay in the following ways:

- They can suppress the response of an assay
- They can enhance the response of an assay
- They can result in an elevated background reading

A small amount of interference from many common substances can be compensated for in the blank designed for a specific assay. To compensate for the interference, the protein samples for the standard curve must be diluted in the same buffer as the protein being assayed.

Often, interfering substances can overwhelm the assay, making it difficult or impossible to perform. The two most popular assay methods, Lowry- or Bradford-based assays, are both strongly affected by various components found in standard sample buffers. Lowry-based methods are incompatible with reducing and chelating agents; DTT, β-mercaptoethanol, cysteine, EDTA and some sugars while Bradford-based methods are incompatible with most detergents. Unfortunately, many common sample buffers contain both reducing agents and detergents, Laemmli buffer for example.

In these situations, the interfering substance can be removed by a variety of means, of which gel filtration and dialysis are the most common. However both of these methods are time-consuming and can result in diluted protein samples. The Compat-Able™ Protein Assay Preparation Set (page 25) was developed to solve this problem. The Compat-Able™ Reagents render potentially interfering substances virtually invisible to either a Lowry- or Bradford-based assay. These unique reagents dispose of any possible interfering substances in your sample by selectively precipitating out the protein, allowing the non-protein sample components to be removed easily. Precipitated protein is recovered in water or an assay-compatible buffer and then assayed by any method.

In one round of treatment, Compat-Able™ Reagents can remove most any interfering substance, including but not limited to:

- Laemmli Buffer
- 3.0 M Tris
- 20% glycerol
- 4% SDS
- 3.6 M magnesium chloride
- 1.25 M sodium chloride
- 350 mM dithiothreitol (DTT)
- 5% Triton® X-100
- 5% Tween®-20
- 125 mM sodium citrate
- 200 mM glucose
- 200 mM sodium acetate
- 5% β-mercaptoethanol
- 200 mM EDTA
- 1.0 M imidazole

If concentrations of these or other interfering components exceed this level, more than one round of pre-treatment can be performed.

Total Protein Assays — Overcoming Interfering Substances

BCA™ Protein Assay – Reducing Agent Compatible

The BCA™ Assay is always compatible with more detergents, buffers/salts and solvents than any other colorimetric protein assay. Now it's compatible with reducing agents at concentrations routinely used in protein sample buffers!

The BCA™ Assay provides one of the most accurate measurements of protein concentration in biological samples available. Although the BCA™ Assay is compatible with more detergents, buffers/salts and solvents than any colorimetric protein assay, the presence of disulfide reducing agents, including dithiothreitol (DTT) and 2-mercaptoethanol interferes with the assay (Figure 1). The BCA™ Protein Assay Kit – Reducing Agent Compatible (Product # 23250) from Pierce provides all the advantages of the original BCA™ Assay as well as compatibility with reducing agents at concentrations routinely used in protein sample buffers (Figures 1 and 3).

References

Smith, P.K., *et al.* (1985). Measurement of protein using bicinchoninic acid. *Anal. Biochem.* **150**, 76-85.

Figure 1A
Standard BCA™ Protein Assay

Figure 1B
BCA™ Protein Assay – Reducing Agent Compatible

Figure 1. Stylized comparison of reducing agent compatibility of standard BCA™ Protein Assay with the BCA™ Protein Assay – Reducing Agent Compatible. Reducing agents (> 1 mM) interfere with the standard BCA™ Protein Assay by artificially increasing the color intensity (Figure 1A). The color intensity produced by the BCA™ Protein Assay – Reducing Agent Compatible is unaffected by the presence of reducing agents (Figure 1B). All samples contained 5 mM DTT and BSA standards at the following concentrations: Tube 1: 0 µg/ml, Tube 2: 125 µg/ml, Tube 3: 250 µg/ml, Tube 4: 500 µg/ml, Tube 5: 750 µg/ml, Tube 6: 1,000 µg/ml, Tube 7: 1,500 µg/ml and Tube 8: 2,000 µg/ml.

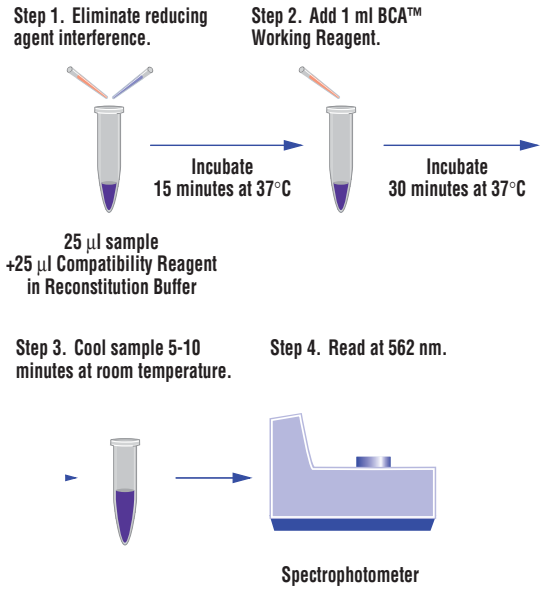


Figure 2. BCA™ Protein Assay – Reducing Agent Compatible protocol.

Highlights:

- Compatible with up to 5 mM DTT, 35 mM 2-mercaptoethanol or 10 mM TCEP
- No protein precipitation required
- Linear working range: 125-2,000 µg/ml
- Sample volume: 25 µl
- Compatible with most ionic and nonionic detergents
- Significantly less protein:protein variation than coomassie (Bradford)-based methods
- Colorimetric method; measure at 562 nm
- Easy-to-use protocol (Figure 2)

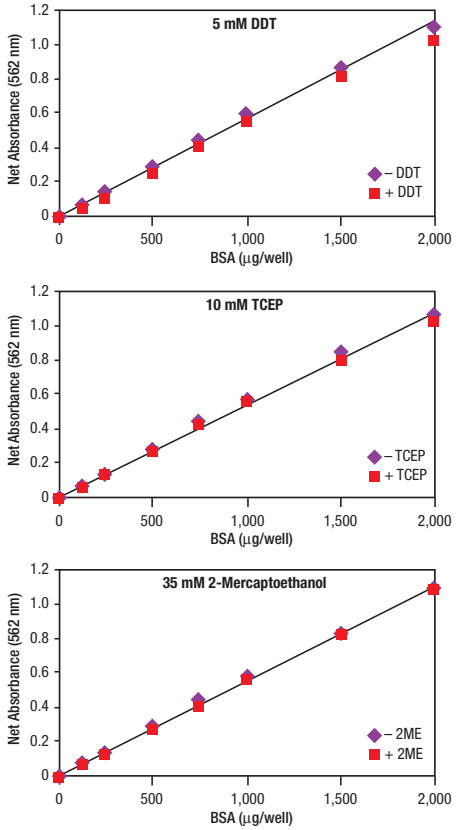


Figure 3. BCA™ Protein Assay – Reducing Agent Compatible produces a linear standard curve in the presence of reducing agents. Color response curves for BSA after treatment with Reducing Agent Compatible Reagent in the presence and absence of 5 mM DTT, 35 mM 2-mercaptoethanol and 10 mM TCEP.

Ordering Information

Product #	Description	Pkg. Size
23250	**BCA™ Protein Assay Kit – Reducing Agent Compatible** *Sufficient reagents to perform 250 standard tube assays.*	Kit
	Includes: BCA™ Reagent A	250 ml
	BCA™ Reagent B	25 ml
	Compatibility Reagent	10 x 20 mg
	Reconstitution Buffer	15 ml
	Albumin Standard (2 mg/ml)	10 x 1 ml ampules

Total Protein Assays — Overcoming Interfering Substances

Compat-Able™ Protein Assays

Excellent choice for use with samples prepared for 1D or 2D electrophoresis.

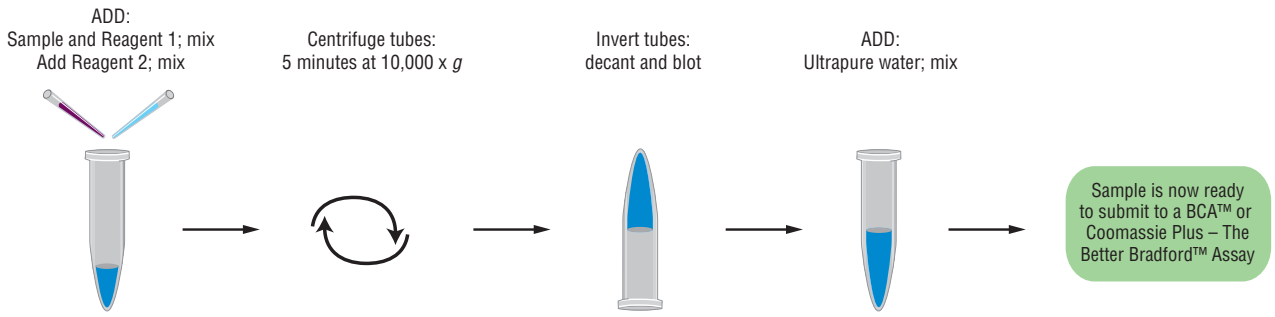
These kits pair Pierce BCA™ and Coomassie Plus – The Better Bradford™ Assays, recognized around the world as the best detergent- and reducing agent-compatible assays (respectively) for total protein analysis, with a great sample preparation reagent. These unique reagents dispose of any interfering substances in your sample by selectively precipitating the protein, allowing the nonprotein components to be removed easily. Precipitated protein is recovered in water and assayed with the BCA™ Protein Assay or Coomassie Plus – The Better Bradford™ Assay.

Highlights:

- Ready-to-use sample preparation reagents save time and effort
- Four-step protocol takes less than 10 minutes to complete
- Room temperature-stable sample preparation reagents can be stored on your bench top so they won't get lost in the cold room or hidden in the lab refrigerator
- Precipitates protein out of solution, leaving potentially interfering substances to be decanted away without dialysis or gel filtration, saving time and avoiding sample loss or dilution
- Easily adaptable to pre-treatment of many samples at one time
- Adaptable to both a test tube and microcentrifuge tube sample preparation protocol, to allow for 50 µl or 100 µl sample volumes
- Sample prep reagents are available with the BCA™ or Coomassie Assays or sold separately

Ordering Information

Product #	Description	Pkg. Size
23229	**BCA™ Compat-Able™ Protein Assay Kit**	Kit
	Contains one each of the following:	
	Product # 23227, BCA Protein Assay Kit	
	Sufficient reagents to perform 250 standard tube assays or 2,500 microplate assays.	
	BCA™ Reagent A	2 x 250 ml
	BCA™ Reagent B	25 ml
	BSA Standards (2 mg/ml)	10 x 1 ml
	Product # 23215, Compat-Able™ Protein Assay Preparation Reagent Set	
	(see description below)	
23239	**Coomassie Plus Compat-Able™ Protein Assay Reagent Kit**	Kit
	Contains one each of the following:	
	Product # 23236, Coomassie Plus Protein Assay Reagent Kit	
	Sufficient materials for 630 standard assays, 950 microassays or 3,160 microplate assays.	
	Coomassie Plus Reagent Formulation	950 ml
	BSA Standards (2 mg/ml)	10 x 1 ml
	Product # 23215, Compat-Able™ Protein Assay Preparation Reagent Set	
	(see description below)	
23215	**Compat-Able™ Protein Assay Preparation Reagent Set**	Kit
	Two-reagent set with sufficient material to pre-treat up to 500 samples prior to total protein assay.	
	Compat-Able™ Protein Assay Preparation Reagent 1	250 ml
	Compat-Able™ Protein Assay Preparation Reagent 2	250 ml



Compat-Able™ Protein Assay protocol. Make almost any protein sample compatible with the BCA™ or Coomassie Plus – The Better Bradford™ Assays in four simple steps.

Total Protein Assays

Modified Lowry Protein Assay

Although the mechanism of color formation for the Modified Lowry Protein Assay is similar to that of the BCA™ Protein Assay, there are several significant differences between the two.

In 1951 Oliver H. Lowry introduced this colorimetric total protein assay method. It offered a significant improvement over previous protein assays and his paper became one of the most cited references in the life science literature. The Modified Lowry Protein Assay uses a stable reagent that replaces two unstable reagents described by Dr. Lowry. The Modified Lowry assay is easy to perform because the incubations are done at room temperature and the assay is sensitive enough to allow the detection of total protein in the low microgram per milliliter range. Essentially, the Modified Lowry protein assay is an enhanced biuret assay involving copper chelation chemistry.

Chemistry of the Modified Lowry Protein Assay

Although the mechanism of color formation for the Modified Lowry Protein Assay is similar to that of the BCA™ Protein Assay, there are several significant differences between the two. The exact mechanism of color formation in the Modified Lowry Protein Assay remains poorly understood. It is known that the color-producing reaction with protein occurs in two distinct steps. As seen in Figure 1, protein is first reacted with alkaline cupric sulfate in the presence of tartrate during a 10-minute incubation at room temperature. During this incubation, a tetradentate copper complex forms from four peptide bonds and one atom of copper. The tetradentate copper complex is light blue in color (this is the "biuret reaction"). Following the incubation, Folin phenol reagent is added. It is believed that the color enhancement occurs when the tetradentate copper complex transfers electrons to the phosphomolybdic/phosphotungstic acid complex (the Folin phenol reagent).

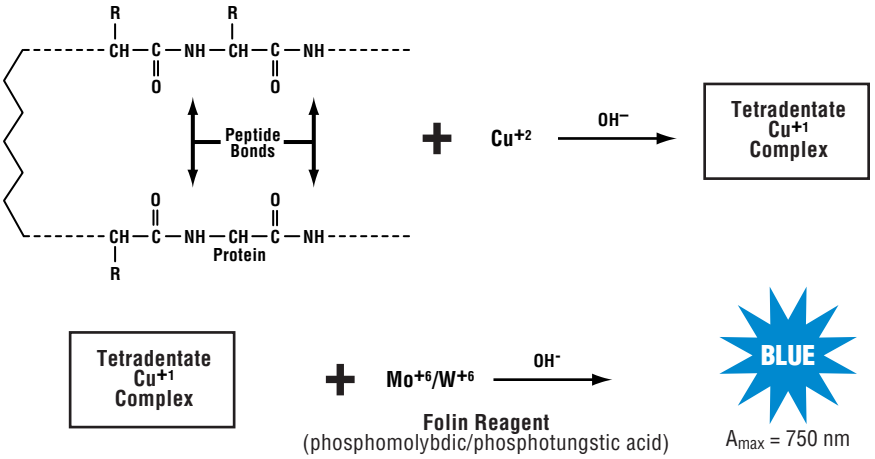


Figure 1. Reaction schematic for the Modified Lowry Protein Assay.

The reduced phosphomolybdic/phosphotungstic acid complex produced by this reaction is intensely blue in color. The Folin phenol reagent loses its reactivity almost immediately upon addition to the alkaline working reagent/sample solution. The blue color continues to intensify during a 30-minute room temperature incubation. It has been suggested by Lowry, *et al.* and by Legler, *et al.* that during the 30-minute incubation, a rearrangement of the initial unstable blue complex leads to the stable final blue colored complex that has higher absorbance.

For small peptides, the amount of color increases with the size of the peptide. The presence of any of five amino acid residues (tyrosine, tryptophan, cysteine, histidine and asparagine) in the peptide or protein backbone further enhances the amount of color produced because they contribute additional reducing equivalents to further reduce the phosphomolybdic/phosphotungstic acid complex. With the exception of tyrosine and tryptophan, free amino acids will not produce a colored product with the Modified Lowry Reagent; however, most dipeptides can be detected. In the absence of any of the five amino acids listed above in the peptide backbone, proteins containing proline residues have a lower color response with the Modified Lowry Reagent due to the amino acid interfering with complex formation.

Advantages of the Modified Lowry Protein Assay

The final blue color is optimally measured at 750 nm, but it can be measured at any wavelength between 650 nm and 750 nm with little loss of color intensity. It is best to measure the color at 750 nm because few other substances absorb light at that wavelength. The amount of light absorbed at 750 nm is directly proportional to the amount of protein in the sample, but the color response curve produced is nonlinear. The sensitivity of the Modified Lowry Protein Assay is greatly enhanced over that of the biuret reagent. The working range of the method extends from 5 to 2,000 mg/ml.

The Modified Lowry Protein Assay demonstrates less protein:protein variability than Coomassie-based assays. When comparing the standard curve responses between BSA and BGG, there is less than a 15% variation in the signal generated with these two standard proteins (Figure 2). The Coomassie Protein Assay demonstrates >30% variation in the signal generated between BSA and BGG (Table 2, page 10).

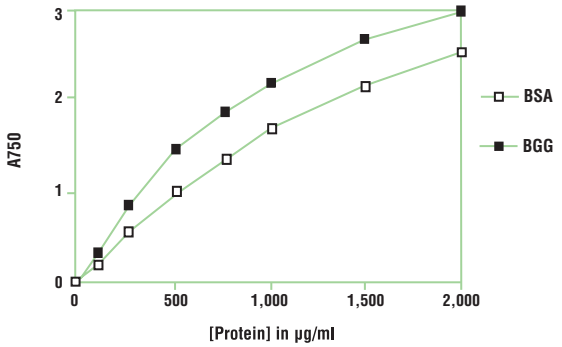


Figure 2. Color response curves obtained with the Modified Lowry Protein Assay Reagent using bovine serum albumin (BSA) and bovine gamma globulin (BGG). The standard tube protocol was performed and the color was measured at 750 nm.

Disadvantages of the Modified Lowry Protein Assay

The Modified Lowry Protein Assay will form precipitates in the presence of detergents or potassium ions. The problem of precipitation caused by the presence of potassium ions in the sample can sometimes be overcome by centrifuging the tube and measuring the color in the supernatant. Most surfactants will cause precipitation of the reagent even at very low concentrations. One exception is sodium dodecyl sulfate (SDS), which is compatible with the reagent at concentrations up to 1% in the sample. Chelating agents interfere by binding copper and preventing formation of the copper peptide bond complex. Reducing agents and free thiols also interfere by reducing the phosphotungstate-phosphomolybdate complex, immediately forming an intensely blue colored product upon their addition to the Modified Lowry Protein Assay Reagent.

General Characteristics of the Modified Lowry Protein Assay

The Modified Lowry Protein Assay Reagent must be refrigerated for long-term storage. If the entire bottle of reagent will be used within one month, it may be stored at room temperature (18-26°C). Reagent that has been left at room temperature for more than one month may produce lower color response, especially at the higher end of the working range. If the reagent has been stored refrigerated, it must be warmed to room temperature before use. Using cold Modified Lowry Protein Assay Reagent will result in low absorbance values.

The protocol requires that the Folin phenol reagent be added to each tube precisely at the end of the 10-minute incubation. At the alkaline pH of the Lowry reagent, the Folin phenol reagent is almost immediately inactivated. Therefore, it is best to add the Folin phenol reagent at the precise time while simultaneously mixing each tube. Because this is somewhat cumbersome, some practice is required to obtain consistent results. This also limits the total number of samples that can be assayed in a single run. If a 10-second interval between tubes is used, the maximum number of tubes that can be assayed within 10 minutes is 60 (10 seconds/tube x 60 tubes = 600 seconds or 10 minutes).

Total Protein Assays

Modified Lowry Protein Assay Reagent

All the accuracy of the Lowry, but modified so it's ready-to-use and stable for at least one year!

Highlights:

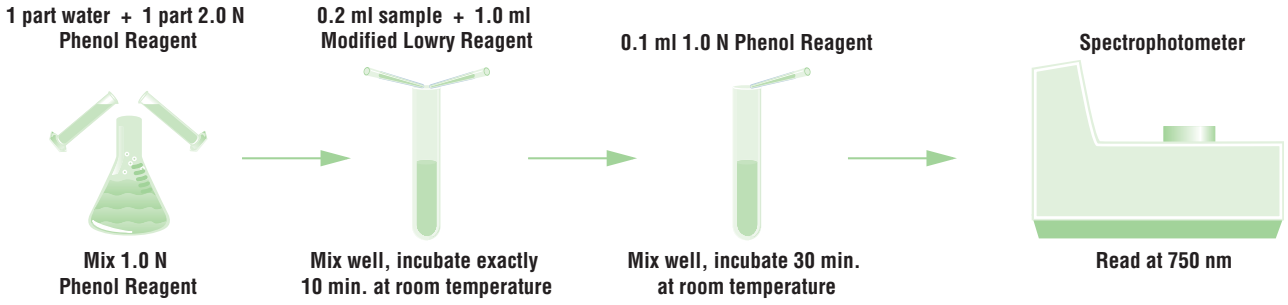
- The most widely cited colorimetric method; read at 750 nm
- Ready-to-use reagent for the loyal Lowry method user
- Preformulated cupric sulfate-tartrate reagent stable for one year at room temperature
- Linear results from 1 to 1,500 µg/ml for BSA
- Adaptable to microplates
- Less protein:protein variation than dye-binding methods

Reference

Lowry, O.H., *et al.* (1951). *J. Biol. Chem.* **193**, 76-85.
Temel, R.E., *et al.* (2003). *J. Biol. Chem.* **278**, 4792-4799.

Ordering Information

Product #	Description	Pkg. Size
23240	**Modified Lowry Protein Assay Kit** *Sufficient reagents to perform 480 standard tube assays or 2,400 microplate assays.*	Kit
	Includes: Modified Lowry Protein Assay Reagent	480 ml
	2 N Folin-Ciocalteu Phenol Reagent	50 ml
	Albumin Standard Ampules (2 mg/ml)	10 x 1 ml



Modified Lowry Protein Assay Reagent protocol.

Total Protein Assays — Amine Detection

o-Phthalaldehyde (OPA) Fluorescent Protein Assay

The Pierce Fluoraldehyde Protein/Peptide Assay is an *o*-phthalaldehyde-based reagent developed to detect minute amounts of protein and peptides. Fluoraldehyde reactions are complete in less than one minute with sensitivity down to 50 ng/ml. While some solutions interfere with protein/peptide measurement at 280 nm, the Pierce Fluoraldehyde Assay is compatible with many substances that interfere with other protein assays, such as detergents and reducing agents. Amine-containing buffers must be avoided, however, when performing assays using this chemistry.

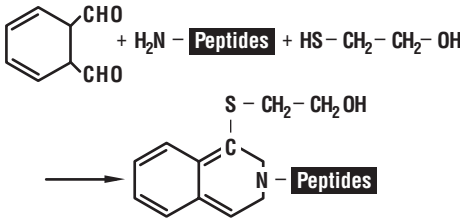
In the standard assay mode, the fluoraldehyde ready-to-use formulation can measure protein concentration in the range of 10 to 500 µg/ml, while the micro-assay working range is 50 ng/ml to 25 µg/ml.

The Pierce Fluoraldehyde Protein/Peptide Assay Reagent requires only 200 µl of sample for use in a microplate assay, saving valuable sample and time. Fluoraldehyde assays require an excitation wavelength of 360 nm and emission wavelength of 455 nm.

OPA will react only with primary amines. When reacted with primary amines in the presence of mercaptoethanol, OPA yields an intense blue colored fluorescent product that has a maximum wavelength of excitation of 340 nm and emission at 455 nm.[1,2] Wavelengths from 330-375 nm have been used for excitation and 436-490 nm for measuring emission. Protein concentrations as low as 50 ng/ml can be measured with an OPA assay. The inherent sensitivity and speed of OPA, along with its broad linear range, makes it a useful protein and peptide assay reagent.

OPA is ideal for assaying peptides that do not contain tyrosine residues, or for other applications in which absorbance at 280 nm cannot be used. Proteins and peptides tested yield linear results over a wide range of concentrations using both standard and microassay protocols.

There is considerable protein:protein and peptide:peptide variation with the OPA assay; therefore, it is best to use a purified sample of the particular protein or peptide as the standard. When this is not possible, the next best option is to use a protein or peptide that gives a response similar to the sample. Alternatively, a commonly accepted standard protein such as bovine serum albumin can be used.



The reaction of *o*-Phthalaldehyde with a primary amine on a peptide in the presence of 2-Mercaptoethanol to form a fluorescent-labeled peptide.

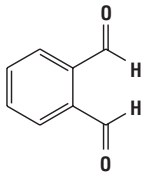
Reducing agents and metal chelators do not interfere with an OPA-based assay, provided they are included in the blanks and standards. In addition, most detergents do not interfere. Any common sample buffers and constituents are also compatible, but primary amines such as Tris or glycine buffers will interfere with OPA and must be avoided. Acetylated and other primary amine-blocked peptides will not give a response with OPA.

References
1. Ogden, G. and Foldi, P. (1987). *LC•GC* **5 (1)**, 28-38.
2. Roth, M. (1971). *Anal. Chem.* **43**, 880-882.

Total Protein Assays — Amine Detection [continued]

Fluoraldehyde™ *o*-Phthalaldehyde Crystals

An easy, economical way to detect amino acids in pre- and post-column chromatographic effluents.



Fluoraldehyde™
o-Phthalaldehyde
M.W. 134.13

Highlights:

- Stable in aqueous solution
- Highly sensitive, low background
- Rapid analysis, no heating required

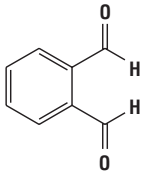
Ordering Information

Product #	Description	Pkg. Size
26015	Fluoraldehyde™ *o*-Phthalaldehyde Crystals	5 g

References
Lindroth, P. and Mopper, K. (1979). *Anal. Chem.* **51**, 1667-1674.
Lee, K.S. and Drescher, D.G. (1979). *J. Biol. Chem.* **254**, 6248-6251.
van Eijk, H.M., *et al.* (1988). *Clin. Chem.* **34**, 2510-2513.
Graser, T.A., *et al.* (1985). *Anal. Biochem.* **151**, 142-152.
Cooper, J.D., *et al.* (1984). *Anal. Biochem.* **142**, 98-102.
Krishnamurti, C.R., *et al.* (1984). *J. Chromatogr.* **315**, 321-331.
Jones, B.N., *et al.* (1983). *J. Chromatogr.* **266**, 471-482.
Lee, H., *et al.* (1979). *Anal. Biochem.* **96**, 298-307.
Chen, R.F., *et al.* (1979). *Biochem. Biophys. Acta* **576**, 440-455.
Jones, B.N., *et al.* (1981). *J. Liq. Chrom.* **4**, 565-586.

Fluoraldehyde™ *o*-Phthalaldehyde Reagent Solution

Excellent sensitivity – an ideal choice when working with limited amounts of purified protein or peptides.



Fluoraldehyde™*o*-Phthalaldehyde Reagent Solution
M.W. 134.13
λex = 340 nm
λem = 455 nm

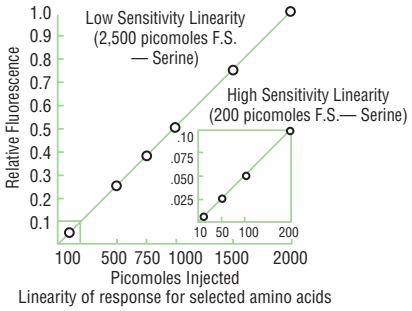
Pierce Fluoraldehyde™ Reagent Solution contains a stabilized, highly purified preparaton of *o*-phthalaldehyde, Brij®-35 Detergent and mercaptoethanol in a specially formulated borate buffer. It is a highly sensitive, ready-to-use reagent solution that exhibits excellent linear response (Figure 1) and offers oustanding shelf life (Figure 2). In addition, when compared to other *o*-phthalaldehyde detection reagents, our solution exhibits decreased background over time and a high signal:noise ratio.

Highlights:

- A ready-to-use, highly sensitive fluorescent pre- or postcolumn reagent for amino acid detection and quantitation
- Provides an accurate measure of both composition and absolute protein/peptide content
- Ready-to-use with no processing needed
- Reacts with all primary amine-containing analytes
- High sensitivity; low background

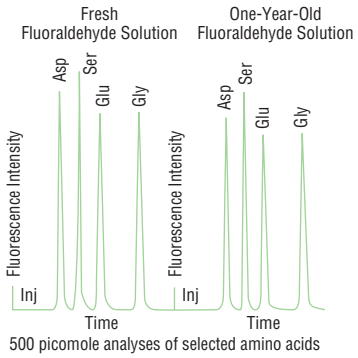
Application Note:

For even greater sensitivity, use a combination of OPA with Fmoc-Chloride with automated pre-column derivatization, detecting both primary and secondary amines. with this application, primary amino acids are first derivatized with OPA, while non-reacted secondary amino acids are then reacted with Fmoc-Chloride, resulting in extraordinary amino acid detection sensitivity and accuracy.[1,2]



Linearity of response for selected amino acids

Figure 1. Excellent linear response. Fluoraldehyde™ Reagent Solution shows excellent linear response, whether in the 2,500 or 200 picomole range.



500 picomole analyses of selected amino acids

Figure 2. Outstanding shelf life. Comparison of fluorescence response of selected amino acids after reaction with recently prepared and one-year-old Fluoreldehyde™ Reagent Solutions.

Ordering Information

Product #	Description	Pkg. Size
26025	Fluoraldehyde™ *o*-Phthalaldehyde Reagent Solution	945 ml

References
1. Godel, H., *et al.* (1992). *LC-GC International* **5**, 44-49.
2. Schuster, R. (1988). *J. Chromatogr.* **431**, 271-284.
Jones, B.N. and Gilligan, J.P. (1983). *American Biotechnology Laboratory*, Dec. Issue, 46-51.
Benson, J.R. and Woo, D.J. (1984). *J. Chromatogr. Sci.* **22**, 386-399.

Specific Protein Assays — Histidine-tagged Proteins

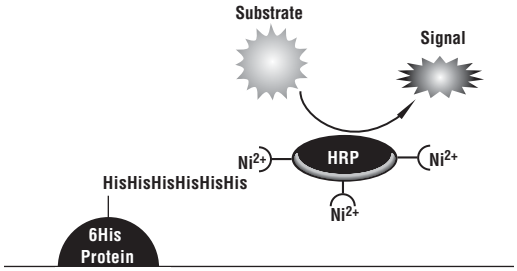
Histidine-Tagged Protein Detection

HisProbe™-HRP Western blotting probe takes advantage of the affinity of histidine for the Ni^{2+} cation.

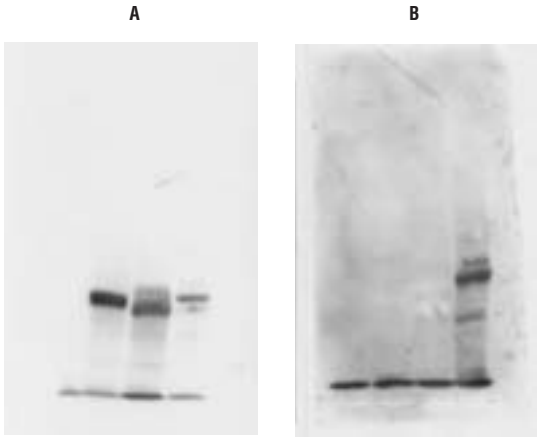
HisProbe™-HRP is a nickel (Ni^{2+})-activated derivative of horseradish peroxidase (HRP). This product has been optimized for direct detection of recombinant histidine-tagged proteins and other histidine-rich proteins. The active ligand is a tridentate chelator that allows Ni^{2+} to be bound in active form for subsequent interaction and detection of target molecules. The active chelator has similar binding capabilities to that reported for iminodiacetic acid, which has long been used for immobilized metal affinity chromatography (IMAC).

Highlights:

- Yields lower background than anti-histidine antibodies
- Pierce HRP is a high-activity enzyme
- Stripping and reprobing is possible
- HisProbe™-HRP (Ni^{2+}) can be used for detection of histidine-tagged proteins



Detection of histidine-tagged fusion proteins with HisProbe™-HRP.



Panel A using HisProbe™-HRP shows high specific binding and low background.
Panel B using anti-polyHis failed to recognize two of the three fusion proteins.

Ordering Information

Product #	Description	Pkg. Size
15165	HisProbe™ HRP†	2 mg
15168	SuperSignal® West Pico HisProbe™ Kit†	Kit
	Includes: HisProbe™-HRP	2 mg
	SuperSignal™ West Pico Chemiluminescent Substrate**	500 ml
	Blocker™ BSA in TBS (10X)	1 x 125 ml
	BupH™ Tris Buffered Saline Packs	10 x 500 ml
	Surfact-Amps® 20 (10%)	6 x 10 ampules

References

Adler, J. and Bibi, E. (2004). Determinants of substrate recognition by the *Escherichia coli* multidrug transporter MdfA identified on both sides of the membrane. *J. Biol. Chem.*, **279**, 8957-8965.

Adler, J. and Bibi, E. (2005). Promiscuity in the geometry of electrostatic interactions between the *Escherichia coli* multidrug resistance transporter MdfA and cationic substrates. *J. Biol. Chem.*, **280**, 2721-2729.

Boulant, S., *et al.* (2003). Unusual multiple recoding events leading to alternative forms of hepatitis C virus core protein from genotype 1b. *J. Biol. Chem.*, **278**, 45785-45792.

Kanaya, E., *et al.* (2001). Zinc release from the CH_2C_6 zinc finger domain of filamentous flower protein from *Arabidopsis thaliana* induces self-assembly. *J. Biol. Chem.*, **276**, 7383-7390.

Robalino, J., *et al.* (2004). Two zebrafish eIF4E family members are differentially expressed and functionally divergent. *J. Biol. Chem.*, **279**, 10532-10541.

Robichon, C., *et al.* (2005). Depletion of apolipoprotein *N*-acyltransferase causes mislocalization of outer membrane lipoproteins in *Escherichia coli*. *J. Biol. Chem.*, **280**, 974-983.

Segawa, H., *et al.* (2005). Reconstitution of GDP-mannose transport activity with purified *Leishmania* LPG2 protein in liposomes. *J. Biol. Chem.*, **280**, 2028-2035.

Sundberg-Smith, L., *et al.* (2005). Adhesion stimulates direct PAK1/ERK2 association and leads to ERK-dependent PAK1 Thr212 phosphorylation. *J. Biol. Chem.*, **280**, 2055-2064.

Wagner, C., *et al.* (2005). Dimerization of NO-sensitive guanylyl cyclase requires the α1 N terminus. *J. Biol. Chem.*, **280**, 17687-17693.

Wann, E., *et al.* (2000). The fibronectin-binding MSCRAMM FnbpA of *Staphylococcus aureus* is a bifunctional protein that also binds to fibrinogen. *J. Biol. Chem.*, **275**, 13863-13871.

Specific Protein Assays — Antibodies

Easy-Titer® IgG and IgM Assay Kits

Simply the fastest, easiest way to quantitate antibodies … ever!

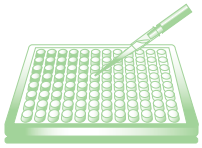
It is no longer necessary to wait or to rely on inaccurate and insensitive UV or colorimetric IgG determination methods. It is not necessary to struggle with the inadequacies of methods that titrate antibody activity. It is even possible to avoid the tedious, time-consuming ELISA approach to determine antibody titer. Easy-Titer® IgG Assay Kits make it possible to detect IgG in less time and with greater specificity and sensitivity than ever before.

Easy-Titer® Assay Kits do not cross-react with antibodies from other species such as bovine antibodies present in the media used to culture antibody-producing hybridoma cells. This remarkable specificity allows the measurement of human IgG concentrations from a variety of sample types such as culture supernatants, ascites or body fluids without first purifying the antibody from other contaminants.

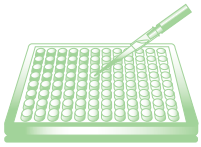
Highlights:

- Easy-to-use particle-based antibody titer determination kit
- Start of assay to recovery of result in less than one hour
- Four times faster than classical ELISA-based protocols
- Convenient design – perform the assay in a 96-well plate and measure the result in a microplate reader
- Measures antibodies from culture supernatants ascites or body fluids
- Measures humanized antibodies and chimeras with intact Fc regions
- No cross-reactivity with Ig from other species

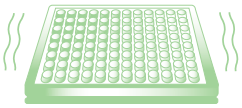
1. Suspend beads



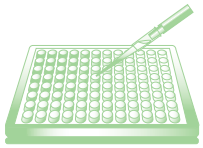
2. Pipette 20 µl beads



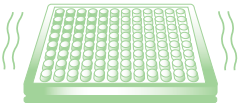
3. Pipette 20 µl sample



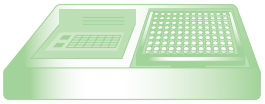
4. Incubate on plate mixer; 5 minutes at room temperature



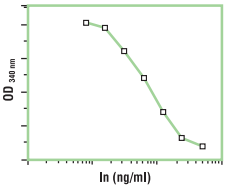
5. Add Blocking Buffer



6. Mix for 5 minutes on plate mixer



7. Read at 405/340 nm



8. Plot Standard Curve; determine concentration of Human IgG or Human IgM

Easy-Titer® IgG and IgM Assay Kit protocol. A simple assay makes for an easy-to-perform assay protocol. Easy-Titer® IgG Assay Kits feature a simple procedure that reduces hands-on time and requires fewer steps that lead to more reproducible results. The entire process can be completed easily in about 30 minutes.

Performance Specifications

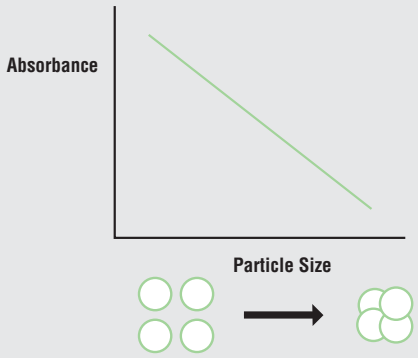
Specificity
- Against all IgG subclasses (human, mouse or rabbit)

Sensitivity
- Detection limit: 15 ng/ml
- Detection range (standard curve): 15 to 300 ng/ml

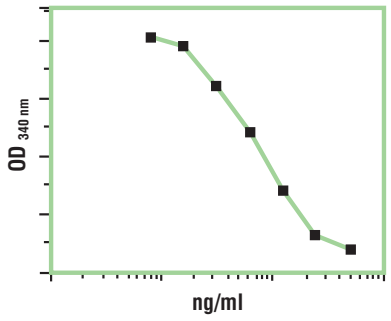
Coefficient of Variation (intra- and interassay): <5%

Reaction time: 10 minutes
- Read results at 340 nm or 405 nm

Standard curve calculations are compatible with software supplied for use with microplate readers.



How the assay works:
- Monodisperse beads sensitized with a specific antibody absorb at 340 and 405 nm
- The beads agglutinate in the presence of human IgG or IgM
- Larger diameter clusters form that absorb less efficiently at 340 and 405 nm
- This decrease in absorbance is proportional to antibody concentration



Typical standard curve for Easy-Titer® Kit. The unknown concentration of IgG is easily determined on a standard curve constructed with serial dilutions of a standard sample.

Ordering Information

Product #	Description	Pkg. Size
23310	**Easy-Titer® Human IgG Assay Kit***	Kit
	Sufficient reagents for 96 tests (87 determinations and one standard curve).	
	Includes: Goat Anti-Human IgG Sensitized Polystyrene Beads [Monodispersed, polystyrene IgG (Fc) sensitized beads are supplied suspended in a phosphate buffer, pH 7.4 and stabilized with BSA and 0.1% sodium azide]	2 ml
	Easy-Titer® Dilution Buffer	30 ml
	Easy-Titer® Blocking Buffer	15 ml
23315	**Easy-Titer® Human IgM Assay Kit***	Kit
	Includes: Goat Anti-Human IgM Sensitized Beads	2 ml
	Easy-Titer® Dilution Buffer	30 ml
	Easy-Titer® Blocking Buffer	15 ml
23300	**Easy-Titer® Mouse IgG Assay Kit***	Kit
	Includes: Goat Anti-Mouse IgG Sensitized Beads	2 ml
	Easy-Titer® Dilution Buffer	30 ml
	Easy-Titer® Blocking Buffer	15 ml
23305	**Easy-Titer® Rabbit IgG Assay Kit***	Kit
	Includes: Goat Anti-Rabbit IgG Sensitized Beads	2 ml
	Easy-Titer® Dilution Buffer	30 ml
	Easy-Titer® Blocking Buffer	15 ml
23325	**Easy-Titer® Human IgG Assay Kit***	Kit
	Includes: Goat Anti-Human IgG Sensitized Beads	2 ml
	Easy-Titer® Dilution Buffer	30 ml
	Easy-Titer® Blocking Buffer	15 ml

Note: An IgG or IgM Standard is not included in these kits.
Select the appropriate standard from the Related Pierce Products listed below.

Related Pierce Products:

IgG Standards for Easy-Titer® Kits

Product #	Description	Pkg. Size
31154	Human IgG, Whole Molecule	10 mg
31146	Human IgM, Whole Molecule	2 mg
31204	Mouse IgG, Whole Molecule	5 mg
31235	Rabbit IgG, Whole Molecule	10 mg

Microplate Accessories

Product #	Description	Pkg. Size
15041	Reacti-Bind™ 96-Well Plates Corner Notch	100 plates
15031	Reacti-Bind™ 8-Well Strip Plates Corner Notch *Includes one strip well ejector per package.*	100 plates
15041	Reacti-Bind™ 96-Well Plates Corner Notch	100 plates
15041	Easy-Titer® Human IgG (Gamma Chain) Assay Kit	Kit

Reference

Brown, M.A., *et al.* (2000). *J. Biol. Chem.* **275**, 19795-19802.

Specific Protein Assays — Proteases

QuantiCleave™ Protease Assay Kits – Colorimetric and Fluorometric

Detects protease as low as 2 ng/ml in less than one hour!

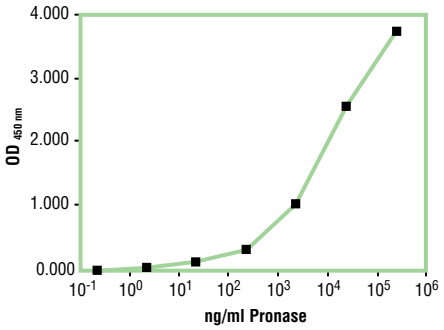
QuantiCleave™ Protease Assay Kits are an ideal choice for performing routine assays necessary during the isolation of proteases, or for identifying the presence of contaminating proteases in protein samples. These protease assays are also ideal for studying pH or temperature vs. activity profiles of purified proteases.

Highlights:

- No corrosive precipitants used
- Entire assay can be run in microplates
- 1,000 times more sensitive, three times faster and uses half the sample of unmodified casein-based protease assays
- Total elapsed time to result – less than one hour
- Measure multiple samples simultaneously in ELISA plate readers
- Time/temperature/pH easily manipulated to optimize sensitivity

The colorimetric QuantiCleave™ Protease Assay Kit uses fully succinylated casein as substrate for this assay. Hydrolysis of this readily soluble casein substrate in the presence of protease results in the release of peptide fragments with free aminoterminal groups. Evidence of protease activity is obtained by reaction of these peptides with trinitrobenzene sulfonic acid (TNBSA), followed by measurement of the absorbance increase that is due to the formation of yellow colored TNB-peptide adducts. A standard protease is provided, allowing you to determine the concentration of protease in samples undergoing analysis.

The QuantiCleave™ Fluorescent Protease Assay Kit is based on a FITC-labeled casein. This sensitive assay can be used in either FRET or FP modes. For more information, refer to the Pierce web site.



Sensitivity of the colorimetric QuantiCleave™ Protease Assay.

Ordering Information

Product #	Description	Pkg. Size
23263	**QuantiCleave™ Protease Assay Kit** *Sufficient material for 250 assays.* Includes: Succinylated Casein (supplied as a lyophilized salt-free powder) 2,4,6-Trinitrobenzene sulfonic acid (TNBSA) TPCK Trypsin standard (40 BAEE units/mg) BupH™ Borate Buffer Pack (makes 500 ml)	Kit 5 x 10 mg 2 ml 50 mg 1 pack
23266	**QuantiCleave™ Fluorescent Protease Assay Kit** *Sufficient material for at least 1,000 assays in a 96-well format.* Includes: FITC-Casein, Lyophilized TPCK Trypsin BupH™ Tris Buffered Saline	Kit 2.5 mg 50 mg 1 pack
23267	**FITC-Casein**	2.5 mg (1,000 assays)

Reference

Rao, S.K., *et al.* (1997). *Anal. Biochem.* **250(2)**, 222-227.

Specific Protein Assays — Glycoproteins

Glycoprotein Carbohydrate Estimation Kit

Direct approach to the estimation of carbohydrate content in proteins.

Highlights:

- Enables quick and easy identification of an unknown protein sample as a glycoprotein
- Estimates the percent carbohydrate content of a glycoprotein when run against a set of glycoprotein standards with known carbohydrate content
- Complementary to electrophoresis, Western blotting and ELISA-based procedures often used to detect glycoprotein
- Determines carbohydrate content in three easy steps: (1) oxidize, (2) react and (3) read
- Entire assay performed in less than 75 minutes
- All you need is this kit, a microplate and a plate reader to determine carbohydrate content

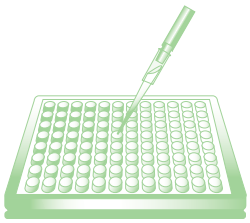
Ordering Information

Product #	Description	Pkg. Size
23260	**Glycoprotein Carbohydrate Estimation Kit** *Sufficient reagents for 250 microplate assays or 60 standard test tube assays.*	Kit
	Includes: Sodium *meta*-Periodate	500 mg
	Glycoprotein Detection Reagent	500 mg
	Glycoprotein Assay Buffer	250 ml
	Negative Controls:	
	Lysozyme and BSA	2.5 mg each
	Positive Controls:	
	Ovalbumin	2.5 mg
	Apo-Transferrin	2.5 mg
	Fetuin	0.25 mg
	α_1-Acid Glycoprotein	0.25 mg
23259	**Lyophilized Glycoprotein Standards Set**	Set
	Includes: Negative Controls:	
	Lysozyme and BSA	2.5 mg each
	Positive Controls:	
	Ovalbumin	2.5 mg
	Apo-Transferrin	2.5 mg
	Fetuin	0.25 mg
	α_1-Acid Glycoprotein	0.25 mg
23262	**Glycoprotein Detection Agent**	1 g

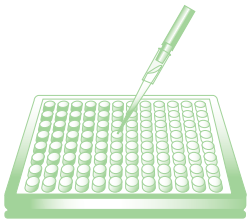
Assay Principle

The protein sample under analysis is oxidized and reacted with the exclusive Glycoprotein Detection Reagent. The resulting colored complex is read at 550 nm. From the absorbance of the resulting complex at 550 nm the approximate percentage of carbohydrate in the glycoprotein under analysis can be estimated.

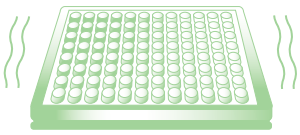
1. Add 50 µl of protein standard or sample to each well.



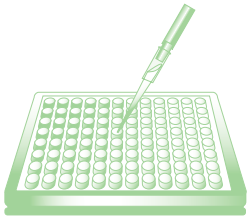
2. Add 25 µl of 10 mM Sodium *meta*-Periodate in assay buffer.



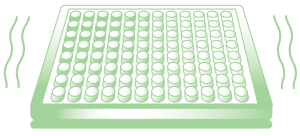
3. Mix and incubate for 10 minutes at room temperature (RT).



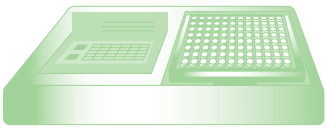
4. Add 150 µl of a 0.5% solution of Pierce Glycoprotein Detection Reagent in 1.0 M NaOH.



5. Mix and incubate at RT for 60 minutes.



6. Read the plate in a microplate reader at 550 nm. Interpolate the results of the unknown with the results of the standard proteins.



The Phosophoprotein Phosphate Estimation Assay microplate protocol.

Specific Protein Assays — Phosphoproteins

Phosphoprotein Phosphate Estimation Kit

Get some basic questions about your target protein answered without having to perform a Western blot.

Pierce introduces a novel protein characterization tool that gives today's protein analyst the ability to quickly and reliably determine whether a purified target protein is phosphorylated and, if so, the extent of phosphorylation compared to a phosphoprotein of known phosphorus content. This easy-to-perform assay is specific for estimating phosphoserine or phosphothreonine post-translational modifications and has been adapted to both a tube and convenient microplate format. The Phosphoprotein Phosphate Estimation Assay provides answers that a traditional Western blot simply cannot, and you can get answers about five times faster, too.

Unique advantage of the assay chemistry

The specificity of this assay toward seryl and threonyl phosphate ester modifications can indirectly "detect" a phosphotyrosine modification should the result of the assay be negative. A negative result on a pure protein preparation can suggest that the protein is not phosphorylated or that the protein is, in fact, phosphorylated, but modified by way of the tyrosyl side chains. Further Western blot analysis can verify which conclusion is correct.

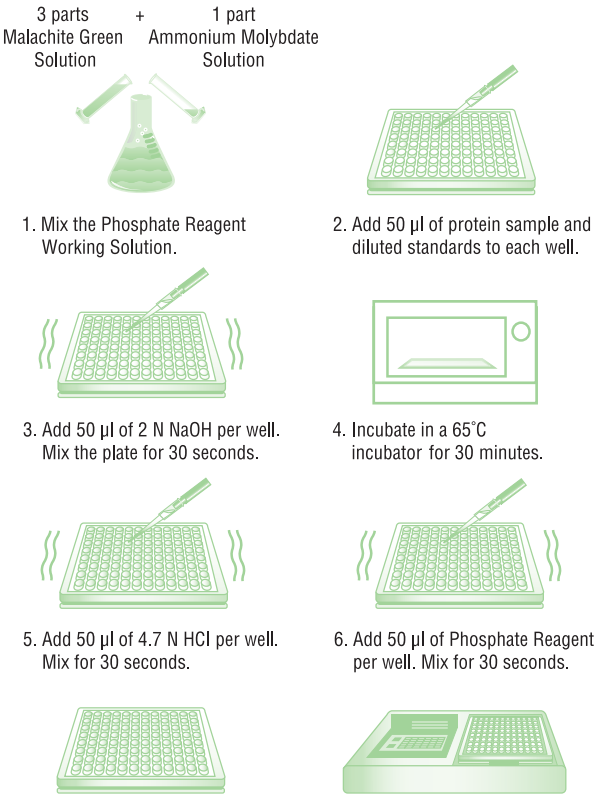
In addition, the Phosphoprotein Phosphate Estimation Assay Kit can also be used to determine the amount of a purified known phosphoprotein in a sample. A standard curve can be constructed using a purified preparation of the known protein.

Highlights:

- Easy-to-prepare working reagent
- Colorimetric detection
- Use as qualitative or semi-quantitative assay
- Test tube or microplate assay option
- Estimate extent of phosphoserine/phosphothreonine modification
- Calculate the moles of phosphate (phosphorus) per mole of purified protein
- Use as quantitative assay for known pure phosphoproteins
- Results in about one hour
- Room temperature stability of kit components – saves refrigerator and freezer space

Assay Principle

The Phosphoprotein Phosphate Estimation Assay is based on the alkaline hydrolysis of phosphate from seryl and threonyl residues in phosphoprotein and the quantification of the released phosphate by the use of malachite green and ammonium molybdate.



The Phosophoprotein Phosphate Estimation Assay microplate protocol.

Ordering Information

Product #	Description	Pkg. Size
23270	**Phosphoprotein Phosphate Estimation Kit** *Sufficient reagents for 20 x 96-well microplate assays or 500 test tube assays.* Includes: Ammonium Molybdate Solution Malachite Green Solution Phosvitin Positive Control BupH™ Tris Buffered Saline	Kit 25 ml 75 ml 1 mg 1 pack

Related Pierce Products:

Product #	Description	Pkg. Size
24550	**GelCode® Phosphoprotein Staining Kit**	Kit

For more product information, or to download a product instruction booklet, visit *www.piercenet.com/path95n.*

Phosphoprotein Detection Reagent and Kit

Novel chemistry enables specific detection of phosphorylated protein.

PhosphoProbe™-HRP is an iron (Fe^{3+})-activated derivative of horseradish peroxidase (HRP). PhosphoProbe™-HRP exhibits two distinct binding specificities, one of which is phosphate ($R\text{-}PO_3$)-specific. The other binding specificity is related to a carboxyl-containing binding motif that is common to most proteins and some peptides. This carboxyl motif binding specificity can be used in a total protein detection application. A novel treatment, termed Reactive Chemical Blocking (RCB) using EDC and ethylenediamine, may be used to eliminate this carboxyl-binding motif, thus imparting exclusive specificity toward phosphate groups. PhosphoProbe™-HRP, in conjunction with RCB, is a universal phosphate detection probe. PhosphoProbe™-HRP has been optimized for direct detection of phosphoester molecules such as nucleotides or protein/peptides containing phosphoserine, phosphothreonine and phosphotyrosine.

Ordering Information

Product #	Description	Pkg. Size
15166	PhosphoProbe™-HRP	2 mg
23031	Ethylenediamine Dihydrochloride	10 g
22980	EDC	5 g
22981	EDC	25 g
15167	Phosphorylated Protein Detection Kit	Kit
	Includes: PhosphoProbe™-HRP	2 mg
	EDC	5 g
	Ethylenediamine	10 g
	Tween®-20	1 vial

Peroxide Assay

PeroXOquant™ Quantitative Peroxide Assay Kits

Quickly measure peroxide contamination in various biological samples.

Highlights:

- Fast and easy to use
- Peroxidase independent
- No lipid extraction necessary
- Spectrophotometric analysis
- No heating required

PeroXOquant™ Quantitative Peroxide Assays are the simplest assays for detecting the presence of peroxides in both aqueous and lipid-containing laboratory reagents. The basis of these assays is the complexing of ferric ion (Fe^{2+}) by H_2O_2 in the presence of xylenol orange. Peroxides in the sample oxidize Fe^{2+} to Fe^{3+}, and the Fe^{3+} will form a colored complex with xylenol orange that can be read at 560 nm.

The presence of hydrogen peroxide (H_2O_2) can now be detected to monitor any peroxide contamination that may be harmful to biological samples. When performed on a routine basis, the Pierce PeroXOquant™ Quantitative Peroxide Assay can prevent inadvertent introduction of peroxides into your valuable samples. If the effects of peroxide cannot be avoided in a particular system, these assays will help you assess the risk to your sample.

References

Coutant, F., *et. al.* (2002). *J. Immunol.* **169**, 688-1695.
Goyer, A., *et. al.* (2002). *Eur. J. Biochem.* **269**, 272-282.
Requena, J. (2001). *Proc. Nat. Acad. Sci., U.S.A.* **98**, 69-74.

Ordering Information

Product #	Description	Pkg. Size
23280	PeroXOquant™ Quantitative Peroxide Assay Kit *Aqueous compatible formulation.* Includes: Reagent A (25 mM Ammonium Ferrous Sulfate) Reagent B (125 µM Xylenol Orange in water with Sorbitol)	Kit 2 x 50 ml
23285	PeroXOquant™ Quantitative Peroxide Assay Kit *Lipid-compatible formulation.* Includes: Reagent A (25 mM Ammonium Ferrous Sulfate) Reagent B (125 µM Xylenol Orange in methanol with BHT)	Kit 4 x 25 ml

Comparison of Assay Protocols for Lipid Peroxide Content

PeroXOquant™ Quantitative Peroxidase Assay

1. Mix one volume of Reagent A with 100 volumes of Reagent C to prepare Working Reagent.
2. Add 950 µl of Working Reagent to 50 µl of sample.
3. Incubate at room temperature for 30 minutes.
4. Read at 560 nm (or 595 nm for ELISA plate readers).

Total Time: 35 Minutes

Thiobarbituric Acid Assay

1. Mix 0.1 ml sample, 0.4 ml H_2O and 0.2 ml 7% SDS.
2. Stir gently and add 2 ml 0.1 N HCl.
3. Add 0.3 ml 10% phosphotungstic acid.
4. Incubate 5 minutes at room temperature.
5. Add 1 ml 0.67% thiobarbituric acid (TBA) and acetic acid.
6. Heat 45 minutes at 95˚C.
7. Cool in ice bath.
8. Add 5 ml butanol.
9. Vortex and centrifuge for 15 minutes.
10. Determine lipid peroxide concentration in butonal layer by fluorescence at 515 nm excitation and 553 nm emission.

Total Time: 80-90 Minutes

PIERCE

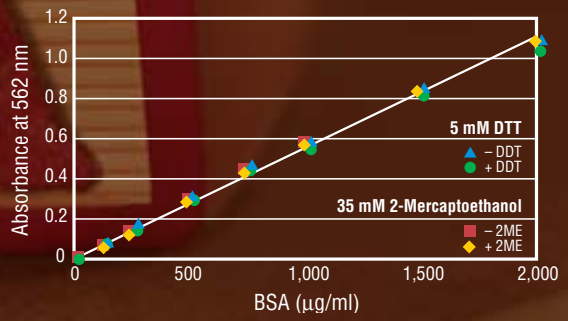
Grasp the Proteome®

Plays well with others.

IT'S BETTER … THE NEWEST BCA™ PROTEIN ASSAY KIT FROM PIERCE IS NOW COMPATIBLE WITH REDUCING AGENTS

Highlights:

- Compatible with up to 5 mM DTT, 35 mM 2-mercaptoethanol or 10 mM TCEP
- Linear working range: 125-2,000 µg/ml
- Sample volume: 25 µl
- Compatible with most ionic and nonionic detergents
- Significantly less (14-23%) protein:protein variation than coomassie (Bradford)-based methods
- Colorimetric method; measure at 562 nm
- Easy-to-use protocol



BCA™ Protein Assay – Reducing Agent Compatible produces a linear standard curve in the presence of reducing agents. Color response curves for BSA after treatment with Reducing Agent Compatible Reagent in the presence and absence of 5 mM DTT and 35 mM 2-mercaptoethanol. For data on 10 mM TCEP, visit our web site.

Ordering Information

Product #	Description	Pkg. Size
23250	BCA™ Protein Assay Kit – Reducing Agent Compatible *Sufficient reagents to perform 250 standard tube assays.*	Kit

Please visit our web site www.piercenet.com *for complete kit components.*

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